Genomic Sequence Resource of *Kabatiella zeae*, the Causative Pathogen of Corn Eyespot Disease

Yafei Wang,^{1,2} Jinai Yao,^{1,3} Xinyao Xia,¹ Zhiqiang Li,¹ Shaoqun Zhou,² Wende Liu,^{1,†} and Hanxiang Wu^{1,†}

¹ State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100193, Beijing, China

² Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, 440307, Shenzhen, China

³ Fujian Key Laboratory for Monitoring and Integrated Management of Crop Pests/Institute of Plant Protection, Fujian Academy of Agricultural Sciences, 350013, Fuzhou, China

Abstract

Kabatiella zeae is the causative pathogen of corn eyespot disease, which is an important leaf disease that damages corn (*Zea mays* L.) worldwide. In this study, we provided an annotated draft of the assembled genome of the *K. zeae* field strain KZ1 through PacBio and Illumina sequencing. The assembled KZ1 genome size is 23,602,820 bp, and its GC content is 50.71%. The completeness of the assembled genome is 97.6% in this study. The assembly obtained in this study has 94 contigs and the length of N₅₀ is 720,243 bp. This study is the first report of the *K. zeae* genome, which contributes to further research on the genetic variation and pathogenic mechanism of this important fungal pathogen.

Corn eyespot disease, which is caused by *Kabatiella zeae*, is one of the most serious leaf diseases of corn (*Zea mays* L.) worldwide. *K. zeae* belongs to order Tuberculariales, class Hyphomycetes, genus *Kabatiella*. It has been pointed out that *Kabatiella* was synonymous with *Aureobasidium* in 1973, and *Aureobasidium zeae* was identified as the pathogen of corn eyespot disease based on fungal classification rules (Dingley 1973). According to morphological and molecular biology studies, *Kabatiella* was considered to be a different genus from *Aureobasidium*; therefore, the pathogenic fungus of corn eyespot was reidentified as *Kabatiella zeae* in 2011 (Seifert and Gams 2011). Corn eyespot disease was first discovered in Japan in 1959 (Narita and Hiratsuka 1959), then successively found in most corn-growing areas around the world (Army et al. 1971; Reifschneider and Army 1979; Santos et al. 2007; Shurtle 1980; Sun et al. 2016). Corn eyespot disease was reported in Jilin Province of China in 1964 (Qi et al. 1966). In 1998, the disease caused a serious loss of total crop yield on farms in Liaoning Province of China (Xu et al. 2000). Thereafter, the disease gradually spread to corn-growing areas in northeast, north, and northwest China, resulting in serious losses in corn production. Corn eyespot disease has recently attracted the attention of researchers because of its serious outbreaks in certain corn-growing areas of China (Sun et al. 2016; Wang et al. 2014; Zhang 2014). However, there is currently no information about the genome of *K. zeae*, which limits the prevention and control of corn eyespot disease. The emergence of new

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Keywords

genome, genomics, Illumina sequencing, *Kabatiella zeae*, PacBio sequencing, pathogenesis

[†]Corresponding authors: W. Liu; liuwende@caas.cn and H. Wu; wuhanxiang@caas.cn

*The e-Xtra logo stands for “electronic extra” and indicates that a supplementary figure is published online.

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Table 1. Genome assembly statistics of *Kabatiella zeae* field isolate KZ1

Variables	Statistics
Genome assembly size (bp)	23,602,820
Number of contigs	94
Contig N ₅₀ (bp)	720,243
Contig N ₉₀ (bp)	158,685
Largest contig length (bp)	1,560,680
GC content (%)	50.71
BUSCO completeness (%)	97.6
Number of genes	8,064
Number of virulence genes	920
Number of CAZymes	341
Number of transmembrane proteins	954
Number of transport proteins	1,346
Number of secreted proteins	639

whole-genome sequencing technologies provides a powerful tool for high-quality genome assembly of important pathogenic fungi (Huo et al. 2021; Zhu et al. 2019). Here, we use PacBio sequencing technology to present the first draft of the genome assembly for *K. zeae* and analyze its genome characteristics, which will help in understanding the pathogenic mechanism of this fungus and developing integrated approaches to control corn eyespot disease.

Strain KZ1 was isolated from field corn leaves in Gongzhuling, Jilin Province, China in October 2019, cultured with potato dextrose agar medium, then subjected to single-spore isolation. Combined with internal transcribed spacer gene sequence analysis and morphological characteristics shown in Supplementary Figure S1, KZ1 was identified as *K. zeae*. The purified fresh fungal tissues were collected for whole-genome sequencing. The Omega Fungal DNA Kit D3390-02 was used to extract fungal genomic DNA, a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, U.S.A.) was used to evaluate the purity and content of DNA, and 0.8% agarose gel electrophoresis was used to test the integrity of the extracted DNA. The PacBio Sequel Single Molecule Real-Time (SMRT) sequencing platform was used to perform whole-genome sequencing of high-quality DNA samples. The SMRTbell library was prepared using the sheared genomic DNA through the SMRTbell sequencing adapter (Pacific Biosciences), and an insert library of approximately 10 kb was sequenced on an SMRT cell using standard methods. The low-quality and short subreads were removed; then, the qualified PacBio subreads were assembled using nextDenovo v2.0-beta.1 (Aimé et al. 2013). All settings were the default except for the following: genome size = 20 M, expected seed depth = 100, and seed cutoff = 6,129. The NEXTflex rapid DNA-Seq kit was used to prepare Illumina sequencing libraries and perform pair-end sequencing on the Illumina HiSeq 4000 platform (parameters were set to 150 bp). The obtained Illumina sequencing data were used to polish the genome assembled with PacBio data through Pilon software (Walker et al. 2014) with default settings and, finally, 8,697 errors were corrected (Walker et al. 2014). The completeness of the assembled genome sequence was evaluated by BUSCO v3, with the gene set shown by Simão et al. (2015). MAKER2 v2.31.9 software was used to predict the gene model in the assembled genome (Holt and Yandell 2011). The parameters were set as follows: organism type = eukaryotic, cpus = 10, max dna len = 100,000, min contig = 1, pred stats = 1, min protein = 30, map forward = 0, keep preds = 1, split hit = 4,000, min intron = 20, single exon = 1, single length = 250, correct est fusion = 0, and evaluate = 1. Basic annotations for the predicted genes were made through five major databases, including Pfam, Swiss-Prot, Gene Ontology, Non-Redundant Protein Database, and Clusters of Orthologous Groups of Proteins (Ashburner et al. 2000; Boutet et al. 2007; El-Gebali et al. 2019; Pruitt et al. 2005; Tatusov et al. 2000). The Kyoto Encyclopedia of Genes and Genomes database was used to further systematically annotate the predicted genes (Kanehisa and Goto 2000).

In this study, PacBio SMRT sequencing produced 2,438,457,264 bp (sequencing depth 103x) and Illumina paired-end sequencing produced 6,308,846,440 bp (sequencing depth 267x) of raw read data. The size of the assembled KZ1 genome was 23,602,820 bp, and the GC content of the genome was 50.71%. The completeness of the assembled KZ1 genome was 97.6%. In this study, 94 contigs were assembled, and N₅₀ was 720,243 bp. Among them, the

contig 44 (JAGFJS010000044.1) was thought to be the mitochondrial genome of KZ1 after NCBI blast. More characteristics of the assembled genome are shown in Table 1. We used MAKER2 software to predict fungal genes and obtained 8,064 genes. Barnmap v0.8 and tRNAscan-SE v1.3.1 software with default settings were used to predict the ribosomal RNA (rRNA) and transfer RNA (tRNA) contained in the genome, and finally obtained 36 rRNAs and 169 tRNAs, respectively (Lowe and Eddy 1997). Carbohydrate-active enzymes (CAZymes) are usually closely related to fungal pathogenicity and affect the interaction between fungi and their hosts (van den Brink and de Vries 2011). The CAZymes identified in the assembled genome by searching against the CAZy database include 182 glycoside hydrolases, 61 glycosyltransferases, 49 auxiliary activities, 46 carbohydrate esterases, 2 polysaccharide lyases, and 1 carbohydrate-binding module (Cantarel et al. 2009). Cytochrome P450 enzymes are a large family of proteins with heme as a prosthetic group. They participate in the metabolism of many endogenous and exogenous substances, including drugs and environmental compounds. In this study, we identified a total of 127 genes in the cytochrome P450 family. It is worth noting that 920 virulence genes related to pathogenesis were predicted through the Database of Fungal Virulence Factors (Lu et al. 2012). After searching against the Pathogen Host Interaction database (E-value cutoff = 1e-05), 2,395 related genes were identified, in which 12 genes were identified as encoding potential effectors (Winnenburg et al. 2006). In addition, 954 transmembrane proteins were identified by searching against the TMHMM database, 1,346 transport proteins were identified by searching against the TCDB database, and 639 secreted proteins were identified by searching against the SignalP database (E-value cutoff = 1e-05 for all searches) (Krogh et al. 2001; Petersen et al. 2011; Saier et al. 2006). These genes are involved in the pathogenic process of fungi and may play important roles in the pathogenesis of *K. zeae*.

We used RepeatMasker v4.0.7 software with default settings to predict the repetitive sequences in the KZ1 genome, and further classified the identified repetitive sequences (Chen 2004). Interspersed repeats, also known as transposon elements (TEs), usually affect the pathogenic variation of various plant fungi (Faino et al. 2016; Raffaele and Kamoun 2012). The total length of the identified repetitive sequence was 11,343 bp, which accounted for 0.05% of the assembled whole genome. We identified 84 LINE retrotransposons, 24 SINE retrotransposons, 18 DNA transposons, 10 LTR retrotransposons, and 1 unclassified TE in the KZ1 genome. In addition, by searching for the telomere repeats at both ends of each assembled contig, telomere repeats (TTAGGG) were finally identified at the 3' ends of contigs 57 (JAGFJS010000057.1) and 94 (JAGFJS010000094.1).

The KZ1 genome assembly provided here is the first available genome of *K. zeae*, which can improve our understanding of the genome characteristics of *K. zeae* and its pathogenic mechanism. To our knowledge, the KZ1 genome assembled here is also the first available genome of the *Kabatiella* genus. The strain sequenced in this study is stored in the State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. PacBio and Illumina sequencing read data have been submitted to NCBI, and the accession number is PRJNA719577. This Whole Genome Shotgun project has been deposited at GenBank under the accession JAGFJS000000000. The version described in this article is version JAGFJS020000000.

Annotation files, supporting files, and the genome analysis result files containing more information are available in the Zenodo repository.

Author-Recommended Internet Resources

Barnmap: <https://github.com/tseemann/barnmap>

BUSCO: <https://busco.ezlab.org>

MAKER2: <http://www.yandell-lab.org/software/maker.html>

RepeatMasker: <http://www.repeatmasker.org>

Zenodo: <https://zenodo.org/record/4777356>

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