BMC Genomic Data

DATA NOTE Open Access

Transcriptomic analysis of maize leaves under different irrigation treatments in field conditions

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Abstract

Objectives As one of the most widely cultivated agricultural crops in the world, maize (*Zea mays* L.) yield is often affected by water stress. In this study, we designed eight different irrigation levels in a field environment, covering a wide range of gradients, and conducted a comprehensive transcriptomic analysis of maize leaves under these eight treatments. The results revealed the molecular mechanisms by which maize responds to drought, optimal irrigation, and excessive irrigation in field conditions. This not only deepens our understanding of maize's response to water stress but also provides valuable genetic resources and theoretical insights for future genetic improvement.

Data description This study designed eight different irrigation levels under field conditions and conducted comprehensive transcriptome sequencing of maize ear leaf tissues. Analysis of the transcriptome data identified differentially expressed genes (DEGs), and principal component analysis (PCA) revealed a clear separation trend among samples under varying water conditions. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses highlighted functional categories associated with water response, cellular metabolism, and growth regulation. These findings provide valuable insights into the molecular mechanisms of maize under drought, optimal irrigation, and over-irrigation conditions, laying a foundation for future genetic improvement efforts.

Keywords Maize (*Zea mays* L.), Irrigation management, Water stress response, Differentially expressed genes, RNA-seq

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Objective

Maize (*Zea mays* L.) is one of the most widely cultivated crop species globally, serving as a staple food, feed, and industrial raw material [1]. With the continuous growth of the global population and the increasing demand for animal feed, the demand for maize is also rising [2]. However, stable and high maize yields depend on relatively stable environmental conditions, and abiotic stresses such as drought, high temperature, salinity, and waterlogging often lead to yield reductions [3–5]. In recent years, with the rapid development of omics technologies such as genomics, transcriptomics, and proteomics, molecular-level studies of crops have become increasingly detailed and in-depth. Among the various abiotic stresses, water stress is particularly critical, as it not only significantly reduces maize yields but also negatively impacts grain quality [6]. However, most omics studies on crop responses to abiotic stress are still conducted under laboratory conditions. In contrast, due to the complex adaptive mechanisms of crops, their performance in field environments often differs from that in laboratory settings [7]. To address this issue, this study aims to explore the molecular mechanisms of maize response to different irrigation levels in field environments through transcriptome sequencing. Comprehensive transcriptome data of maize leaves under different irrigation conditions were obtained, providing valuable genetic resources and theoretical insights for future breeding strategies to improve maize drought resistance and optimize irrigation management in maize production.

This study employed the DNBSEQ platform to perform transcriptome sequencing on maize ear leaf tissues subjected to different irrigation gradients under field conditions [8]. By designing multiple irrigation levels, the study captured the molecular response characteristics of maize under water deficit, optimal irrigation, and over-irrigation conditions, aiming to elucidate the molecular mechanisms of maize under water stress, normal irrigation, and excessive irrigation in realistic field environments. RNA sequencing analysis identified differentially expressed genes (DEGs), revealing significant changes in the expression of genes involved in various metabolic processes and signaling pathways under water stress conditions. These findings provide new insights into the molecular mechanisms of maize in response to drought and over-irrigation in field environments, enrich the genetic resources of maize, and offer a theoretical basis for improving breeding strategies.

Data description

Experimental design and sample collection

This experiment was conducted at the Weishan Irrigation Experiment Station in Liaocheng, Shandong Province, China (36°38′44″N, 116°13′80″E). The trial was carried out in bottomed pits equipped with motorized rain shelters. During rainfall, the rain shelters were extended to cover the pits, and retracted once the rain stopped. Each pit measured 3.3 m in length, 2.0 m in width, and 2.0 m in depth, arranged in two rows oriented east to west, with 12 pits per row, for a total of 24 pits. In this study, the summer maize variety "Zhengdan 958" was selected. "Zhengdan 958" is the main cultivated variety in the Huang-huai-hai region of China. It has strong adaptability, high yield potential and good stress resistance, and can exhibit stable growth characteristics under different environmental conditions. Therefore, we selected this variety. During the entire growth period of summer maize, irrigation was carried out three times, specifically at the jointing stage, tasseling stage, and grain-filling stage. Eight irrigation treatments were established, with water amounts ranging from low to high as 80, 160, 240, 280, 320, 360, 400, and 480 mm, labeled as M1 to M8. Each treatment was repeated three times. Leaf samples were collected from the upper one-third of the ear leaf 10 days after the third irrigation. The samples were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

RNA extraction, library construction, and transcriptome sequencing

Total RNA was extracted from maize leaf samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RNA concentration and purity were determined using a NanoPhotometer spectrophotometer (IMPLEN, CA, USA) by measuring the absorbance ratios at A260/280 and A260/230. RNA integrity was further assessed using 1.2% (w/v) denaturing agarose gels and verified by determining RNA integrity number (RIN) values with the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). For library construction, polyadenylated mRNA was isolated from total RNA using Oligo (dT) magnetic beads. The enriched mRNA was fragmented using a fragmentation buffer, and the resulting RNA fragments were reverse-transcribed into cDNA using random hexamer primers. After synthesis of the double-stranded cDNA, end-repair and A-tailing were conducted, followed by ligation of sequencing adapters. The cDNA libraries were then purified and amplified by PCR. Library quality was evaluated using the Agilent 2100 Bioanalyzer. High-quality libraries were sequenced on the DNBSEQ platform (MGI, Shenzhen, China), generating paired-end reads of 150 base pairs.

Gene expression analysis

Raw sequence data quality was assessed using FastQC (v0.12.1) to ensure high-quality reads. Low-quality bases, adapter sequences, and poly-N regions were removed

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data file 1	Transcriptome profiles of maize under 8 different irrigation treatments	Portable document format (.pdf)	Figshare (https://doi.org/10.6084/m9.figshare.27823071)[12]
Data file 2	GO analysis	ZIP file (.zip)	Figshare (https://doi.org/10.6084/m9.figshare.27823071)[12]
Data file 3	KEGG analysis	ZIP file (.zip)	Figshare (https://doi.org/10.6084/m9.figshare.27823071)[12]
Data set 1	Maize transcriptome sequencing	Fastq file (fastq.gz)	CNGB (https://doi.org/10.26036/CNP0006534) [17]

using fastp v0.12.4 to generate clean data for downstream analyses [9]. The Phred quality score (Q20 and Q30) and GC content of the clean reads were calculated to further confirm data integrity. For alignment, the clean reads were mapped to the maize reference genome B73 (ZmB73_Ref-Gen_v4) using HISAT2 (v2.0.5) with default parameters [10]. FeatureCounts (v1.6.4) was used to count the number of fragments mapped to each gene, and transcript per million (TPM) values were calculated to represent gene expression levels in each sample [11]. Principal component analysis (PCA) was used to assess the clustering of transcriptomes under different treatments, revealing significant differences among the eight treatment groups (Table 1) [12]. To identify DEGs, DESeq2 was employed, normalizing read counts to account for sequencing depth and gene length [13]. A log2 fold change ($|\log2FC| \geq 1$) and a *p*-value adjusted to a false discovery rate ($FDR \leq 0.05$) were used to determine significant differential expression. Gene Ontology (GO) [14] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses (https://www.genome.jp/kegg/) were conducted on the identified DEGs [15]. Through comparative analysis of the treatments, we identified significantly enriched pathways in the GO (Table 1) [12] and KEGG (Table 1) [12] enrichment analyses. The GO enrichment analysis revealed pathways related to photosynthetic processes, secondary metabolic processes such as terpenoid biosynthetic process, isoprenoid biosynthetic process, phenylpropanoid biosynthetic process, lignin metabolic process, and antibiotic catabolic process. Additionally, primary metabolic processes including proline biosynthetic process and lipid oxidation were enriched, along with signaling and regulatory pathways such as the abscisic acid-activated signaling pathway and hormone catabolic process. The KEGG pathway enrichment analysis identified several categories of biological processes. Pathways related to photosynthetic processes included Photosynthesis, Photosynthesis - antenna proteins, and Carbon fixation by Calvin cycle. Secondary metabolic processes were represented by Phenylpropanoid biosynthesis, Flavonoid biosynthesis, Diterpenoid biosynthesis, Brassinosteroid biosynthesis, and Sesquiterpenoid and triterpenoid biosynthesis. Primary metabolic processes included Ascorbate and aldarate metabolism, Starch and sucrose metabolism, Amino sugar and nucleotide sugar metabolism, and Nitrogen metabolism. Additionally, signaling and regulatory pathways such as the MAPK signaling pathway - plant, NF-kappa B signaling pathway, Toll and Imd signaling pathway, and the Abscisic acid-activated signaling pathway were enriched. Notably, among all treatment comparisons, photosynthesis-associated pathways exhibited the most significant enrichment (Table 1) [12]. As a critical process influencing biomass accumulation in crops [16], photosynthesis encompasses key steps in light harvesting, photosynthetic processes, responses to light conditions, photomorphogenesis, and photoreceptor signaling. These processes are essential for energy acquisition and play a pivotal role in determining maize yield.

These findings highlight changes in photosynthesis-related pathways that occur in maize from moderate to over-irrigation. By capturing the molecular responses of maize under different irrigation levels, especially the regulation of photosynthesis under field conditions, this study provides new insights into gene regulatory mechanisms affected by water resources. Ultimately, these results enhance our understanding of how water stress affects maize at the genetic level, providing valuable knowledge for future crop improvement strategies.

Limitations

- Regional and Environmental Limitations: This study was conducted in Liaocheng, Shandong Province, under specific climatic and soil conditions, limiting the generalizability of the findings to other regions with different environments. Multi-location trials would enhance broader applicability.
- Experimental Design and Genetic Background: The study used eight irrigation treatments but did not cover extreme drought or over-irrigation scenarios. It focused solely on the summer maize variety "Zhengdan 958," potentially limiting the conclusions. Future research should include more maize varieties and irrigation conditions.
- Transcriptome Analysis Limitations: Limited sampling time points and reliance on a reference genome may have caused incomplete gene identification. Different bioinformatics tools and incomplete gene annotations might have affected

data interpretation. Expanding sampling periods and integrating multi-omics could improve future studies.

Abbreviations

PCA	Principal Component Analysis
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
DEGs	Differentially Expressed Genes

Author contributions

PFC, KHJ, JY, XGL, HZY, and HZ conceptualized and designed the study. ZWW performed the data analysis. RZL was responsible for tissue sampling. YXL drafted the initial manuscript. PFC provided critical revisions and finalized the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding

This research was supported by the Horizontal Research Project "Key Engineering Technologies for Smart Agriculture" (K23LD90) and the College Student Innovation and Entrepreneurship Training Program (CXCY2023254).

Data availability

The data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0006534.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 20 November 2024 / Accepted: 27 January 2025
Published online: 19 March 2025

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