minor modifications. The 2.0 mm tissue slices were cut transversely from the center of the okra pod, and five pods were taken from each group of treatments to obtain the tissues according to the above method. The tissue slices were placed in a glass petri dish and immersed in a 15% phloroglucinol solution for 3min. Concentrated hydrochloric acid (2.0 mL) was added to discolor the okra flake tissue for 5 min, and the lignified tissue and lignin particles quickly turned red. The photos were taken by 5-megapixel Canon camera.

### 2.5. Determination of NO, ethylene, ABA, $H_2O_2$, MDA contents and NOS, SOD, POD, and CAT activities

NO content was measured with Plant NO ELISA Kit (Catalog number: A012-1, purchased from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd). Ethylene content was measured with Plant Ethylene ELISA Kit (Catalog number: 2 Pl-KMLJ91119p, purchased from Nanjing Camilo Biological Engineering Co., Ltd.). ABA content was measured with Plant ABA ELISA Kit (Catalog number: H251, purchased from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd.). $H_2O_2$ content was measured with Plant $H_2O_2$ ELISA Kit (Catalog number: A064-1, purchased from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd). NOS activity was determined by Plant NOS ELISA Kit (Catalog number: A014-2, purchased from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd). CAT activity was determined by Plant CAT ELISA Kit (Catalog number: A007-1, purchased from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd).

The activities of SOD and POD, and the content of MDA were measured according to the method described by Feng et al. (2008). The determination of each set of data contains three or more biological replicates to reduce random measurement errors and ensure the reliability of the data.

### 2.6. Determination of lignin content

Lignin content was determined according to the method described by Liu et al. (2018) with some modifications. The okra tissue was frozen with liquid nitrogen and ground well, mixed with 5.0 mL extracting buffer (100 mM $K_2HPO_4/KH_2PO_4$, 0.5% TritonX-100, 0.5% PVP, pH = 7.8), slowly stirred for 30 min, and centrifuged at 6000 rpm for 20 min. The precipitate was immersed with methanol for 30 min, then dried at 80 °C for 12 h. Then, 10.0 mg of the residue was transferred into a new tube, and 1.0 mL of 2M HCl and 0.1 mL of thioglycolic acid were added in. The mixture was heated at 100 °C for 8 h, cooled on ice and centrifuged at 15000 rpm for 20 min at 4 °C. The precipitate was washed with distilled water and resuspended in 2.0 mL of 1M NaOH. After gently stirred at room temperature for 18 h, the solution was centrifuged at 1000 rpm for 20 min, and 0.5 mL of the supernatant was transferred to a new tube. 100 μL of HCI was added to the tube, and the lignin-thioglycolic acid mixture was precipitated at 4 °C for 4 h. The mixed solution was centrifuged at 15000 rpm for 20 min, and the precipitate was dissolved in 1.0 mL of 1M NaOH. The absorbance was tested at 280 nm and 1M NaOH was used as the blank control.

The determination of each set of data contains three or more biological replicates to reduce random measurement errors and ensure the reliability of the data.

### 2.7. Quantitative real-time PCR (qRT-PCR) analysis

Primers are designed according to gene information sequenced by full-length transcriptome analysis, and primer sequences are listed in Supplementary Table 4. qRT-PCR amplifications were carried out by ABI PRISM 7500 (Fast Real-Time PCR Systems, V2.0.1, USA) using BeyoFast Probe One-Step qRT-PCR Kit (purchased from Shanghai Beyotime Biotechnology Co., Ltd.). Quantification of the gene expression level was measured with comparative $C_T$ method described by Schmittgen and Livak (2008). The tests were done in triplicate and each data represents the average of the above experiments.

### 2.8. Data statistical analysis

All experiments were repeated for three or more times. The measured data in the experiment were processed and plotted using GraphPad Prism 8.0 software, and significance analysis was conducted with Two-Way ANOVA multiple comparison ($P < 0.05$ or $P < 0.01$).

## 3. Results

### 3.1. Sequencing, de novo assembly and analysis of okra full-length transcriptome

$A_{260}$ and $A_{280}$ absorbance values of the total RNA were 1.92 and 1.99, the 28S/18S ratio was 1.80, and the RIN value was 9.11, indicating that the quality of the total RNA was suitable for subsequent analysis, including gene function annotation, classification, and similarity analysis.

Based on HiSeqTM 2500 sequencing platform, a total of 18,109,759 reads were obtained from the transcriptomes of various tissues of okra, with a total base number of 35,014,941,499 bp (35.01 G) (Accession Number: SRR13983395). Among them, the shortest sequence length was 50 bp, the longest sequence length was 239165 bp, and the average sequence length was 1933.48 bp (Supplementary Fig. 1A). However, the original subreads have low quality, high degree of redundancy and the presence of chimeras. By CCS (circular consensus sequence) analysis, the subreads were corrected to obtain a consensus sequence, then isoseq3 was used to remove the primers and the barcodes were detected. Thereafter, the cluster of isoseq3 was used to remove the polyA tail and chimera structure, similar sequences were clustered to generate a FLNC (full-length non-chimera) consensus sequence. Then the polish command of isoseq3 was used to polish and correct the FLNC sequence to produce a non-redundant consistent sequence. Through these steps, high-quality isoform sequences (prediction accuracy greater than 99% and supported by more than two full-length sequences) were obtained. The total number of okra transcript was 49487, the total number of bases was 104723539 bp, of which the shortest base was 96 bp, the longest base was 7691 bp, and the average sequence length was 2116.18 bp (Supplementary Fig. 1B). The high-quality Isoform sequence data of all samples were clustered together by CD-HIT (identify = 98%) to obtain unigene sequence, and the subsequent analysis was based on these unigene sequence. The analysis results show that the total number of unigene sequences was 42521, the total number of bases was 89875559 bp, the shortest base was 96 bp, the longest base was 7691 bp, and the average sequence length was 2113.67 bp (Supplementary Fig. 1C).

Diamond software was used to perform sequence similarity alignment between okra unigene and NR (non-redundant) database. The analysis results showed that 41831 unigenes were found in the NR database with similar sequences, of which 2242 unigenes could be annotated with a length of 300–1000 bp (5.27%), and 17524 unigenes could be annotated with a length longer than 1000 bp (41.21%). Ten species with high sequence similarity were noted in the NR database, including *Gossypium raimondii* (10904, 26.07%), *Gossypium hirsutum* (7558, 18.07%), *Gossypium arboretum* (7361, 17.6%), *Durio zibethyleneinus* (4665, 11.15%), *Gossypium barbadense* (4332, 10.36%), *Theobroma cacao* (2601, 6.22%), *Herrania umbratical* (1512, 3.61%), *Corchorus olitorius* (455, 1.09%), *Corchorus capsularis* (488, 1.07%), *Abelmoschus esculentus* L. cv. Xianzhi (133, 0.32%) (Supplementary Fig. 2).

To obtain the metabolic pathways of okra gene products in cells, unigenes were compared with the KEGG database for annotation of metabolic pathways. The result showed that the unigenes involved in the KEGG metabolic pathway in the okra transcriptome could be divided into 4 major branches, including 'cellular processes' (2598),