Supporting Information

Physiological and molecular level understanding of advanced carbon dots to enhance maize drought tolerance: modulation of photosynthesis and signaling molecules

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Number of figures: 20
Number of tables: 1

Supplementary Text S1: The preparation of N-CDs and PNDs

Briefly, citric acid (1.0507 g, Sigma Aldrich) and ethylenediamine (335 μL, Sigma Aldrich) in ultrapure water (10 mL) were heated at 200 °C for 10 h in Teflon-lined autoclave chamber.[1] Then, 10% polyacrylic acid (1 mL, PAA, Sigma Aldrich) solution was added to the N-CDs solution and heated at 80 °C for 4 h for preparing PNDs.[2] All products were dialyzed in a dialysis bag (MW=500Da) and dried by a vacuum freeze dryer.

Supplementary Text S2: The watering processes

The level of soil moisture was maintained by manual irrigation with ultrapure water. Maize plants were watered once per two days from 9:00–11:00 am (normal growth 200–250 mL, and drought stress 50–100 mL). The soil moisture sensor was used to measure water contents in all pots. Meanwhile, soil moisture of different treatments was randomly verified by soil weighing, and there was no significant difference between the results and those measured by sensor.

Supplementary Text S3: detailed parameters of sensor

Soil Moisture Technical Parameters in TZS–IW Sensor

Moisture unit: %

Moisture content test range: 0–100%

Response time: ≤2 seconds

Relative percentage error: ≤3%

Soil moisture probe working temperature: –40 ℃–80 ℃

Standard cable length: 1.5 m (can be customized according to customer needs, the longest can be up to 1000 meters)

Moisture storage data: 2000 groups

Supplementary Text S4: detailed measurements

ROS measurement

The maize leaves were collected and wiped from each treatment. The leaves were cut into 5 mm diameter discs by using a puncher. These leaf discs were transferred into 2', 7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Sigma Aldrich) dye with 25 μM, then incubated for 30 min without illumination. Leaf discs were placed between a glass

slide and a coverslip. The samples were then observed using a confocal microscope (Nikon A1+ Confocal Superresolution Imaging System, Japan). Parameter settings: 20×wet objective; 488 nm laser excitation; PMT: 500–600 nm.

Pro content measurement

Frozen leaves of maize (50–100 mg) were ground in liquid nitrogen. Maize material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered. The filtrate (2 mL) was reacted with 2 ml acid-ninhydrin (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid) and 2 ml of glacial acetic acid in a test tube for 1 hour at 100 ℃. The reaction mixture was extracted with toluene (4 mL), mixed vigorously with a test tube stirrer for 30 sec. The chromophore containing toluene was extracted from the aqueous phase and the absorbance was read at 520 nm. The proline concentration was determined according to previous research.[3]

ABA content measurement

Fresh roots or shoots (0.4 g) was ground to powders in liquid nitrogen and mixed with isopropanol/hydrochloric acid extract buffer solution (10 mL). After shaking at 4 ℃ for 12 h, the mixture was added with dichloromethane and then shaken in 4 ℃ for 1 h. The reaction mix was filtered using 0.45 μm microfilter. By freeze drying, the dried extracts were dissolved in 200 μL methanol with 0.1% formic acid, following that 200 μL of ultra-pure water was added with 0.1% formic acid. ABA content was determined by high performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS, Thermo Scientific, Germany). The detailed HPLC–MS/MS condition was referred to the method in accordance with Yue et al.[4]

Determination of element content

Maize shoots and roots were ground into powder after drying. 25 mg of powder was placed into digestion tube and dissolved in 10 ml of 60% nitric acid (v/v). After microwave digestion for 2 hours, the reaction mixture was filtered with 0.22 μm microfilter and followed by diluting to 50 mL with first-grade water. The K^+ and Na^+ concentrations were measured by inductively coupled plasma mass spectrometry (ICP–MS) (ICAP–TQ, Thermo Fisher Scientific, Germany). The detailed ICP–MS condition was referred to the method described by Wang et al.[5]

Determination of ATP and NADPH production

The production of NADPH and ATP were as measured by an ELISA kit (Cat No. 2Pl-KMLJ91008p, Cat No. 2Pl–KMLJ91598p). All reagents, samples and standards were prepared according to the manufacturer's protocol, and the results of NADPH and ATP were calculated in accordance with the kit instructions.

Quantitative real-time PCR (qRT–PCR)

qRT–PCR analysis was performed as previously described.[6] Briefly, according to the manufacturer's protocol, total RNA was extracted using the RNA Pure Plant Kit (DNaseI) (CWBIO, China). RNA (1 μg) was transcribed into cDNA by reverse transcription polymerase chain reaction and cDNA synthesis kit (CW Biotechnology Co., Ltd., Jiangsu, China). All genes were quantified using UltraSYBR mixture (CWBIO, China) and CFX96TM optical module real-time PCR detection system (Bio-Rad, China). The details of the reference genes and primers were listed in Supplementary Table S1.

Statistical analysis

Before PCA (Figure S21) analysis, data normalization (by sum) has been done for general-purpose adjustment between CK and PNDs exposure under drought, and data transformation (log transformation) was conducted to make individual features more comparable.[7] Metabolites with VIP $\geq$ 1 or adjusted $P \leq 0.05$ were considered significantly differential metabolites. A variable with a fold change (FC) was defined to measure the change of metabolite in this study. The impact value threshold calculated for pathway identification was set at 0.1, and an enrichment factor of 2 was used. Biological pathway analysis was performed on HPLC–MS/MS data by use of MetaboAnalyst 2.0.

Table S1. Primer sequences used in this study[4, 5, 8]

Primer name	Sequence (5'-3')
M-actin-F	CATGAGGCCACGTACAACTC
M-actin-R	TCATGGCAGTTCATGTATTG
ZmSUT4-F	GCTGCAGAATTCGTCTGGAAACTCTTTGTGGGT
ZmSUT4-R	CGGATCCTCGAGCTCCTGTAACTTTTATTCATTGC T
ZmABA1-F	CTTTTGGTTGGTGCCGATGG
ZmABA1-R	GCCAAACGTCCTAGTATCGC
ZmABA3-F	GGATTTGCCATCACAGCAGC
ZmABA3-R	AGCAGCTACCGTTCCATTTTT
ZmAAO3-F	CTTTGAGACTTTCCGAGCAG
ZmAAO3-R	AGGGAGGATAGGAAGGTAAACA
ZmNCED-F	CTTTGAGACTTTCCGAGCAG
ZmNCED-R	AGGGAGGATAGGAAGGTAAACA
ZmPIP1;1-F	CCCTACTATGTTACGTGGAGTTC
ZmPIP1;1-R	GCGGCATATTACACAATTGGTA
ZmPIP1;2-F	GCTCAAACAGACAAGGACTAC
ZmPIP1;2-R	CAAGATGATGATGATGATGACTCGAAAG
ZmPIP2;4-F	TACCGGAGCAACGCCTAAG
ZmPIP2;4-R	GAAAACAGCAGCGAGCGA
ZmPIP2;5-F	TGTCGTCGTTGGTTGCCT
ZmPIP2;5-R	CACAACAATCACACTAGCTTGGAA
ZmTIP1;1-F	GCTCGCCGCCGTTTAGTTTCT
ZmTIP1;1-R	GACGACTGCTGGTCCAAGGAAG
ZmNIP1;1-F	GGATCTACGGCAGCGACAAGGA
ZmNIP1;1-R	AATGGCGGAACACGGCGAAC
ZmBetProt-F	TCAGGATTTGGTTGGGTTCT
ZmBetProt-R	TGATTTGGGGATG

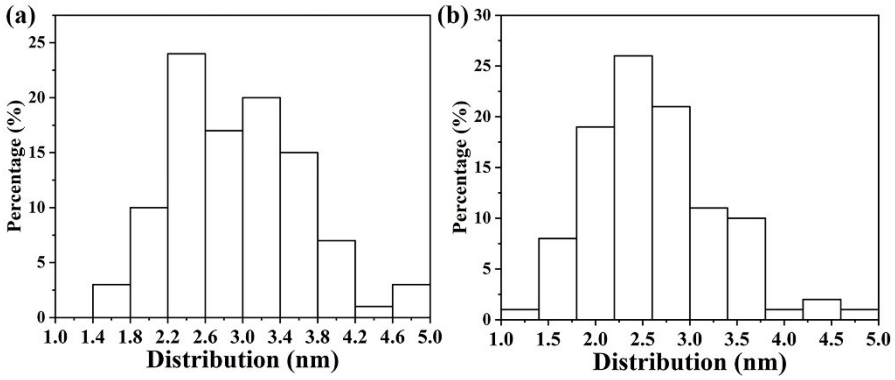

Figure S1. The size distribution of N-CDs (a) and PNDs (b).

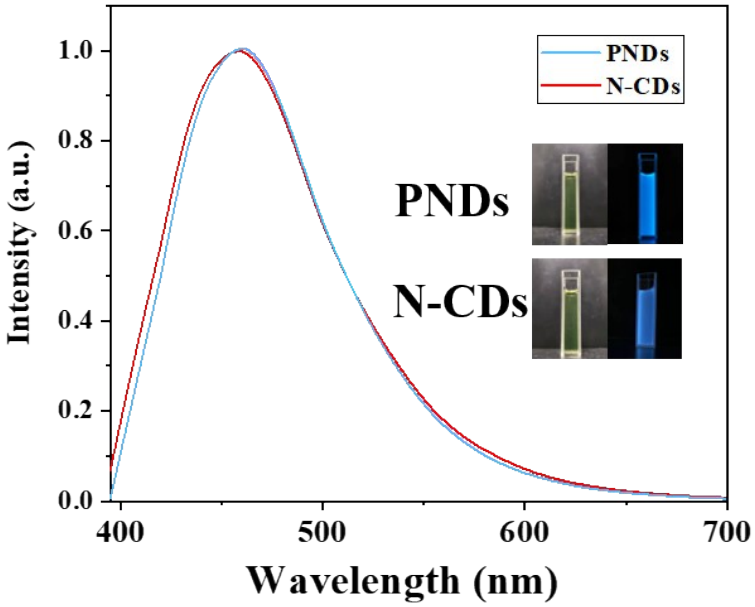

Figure S2. Fluorescence spectra of N-CDs and PNDs (inset: photographs of N-CDs and PNDs under room light and 365 nm irradiation light).

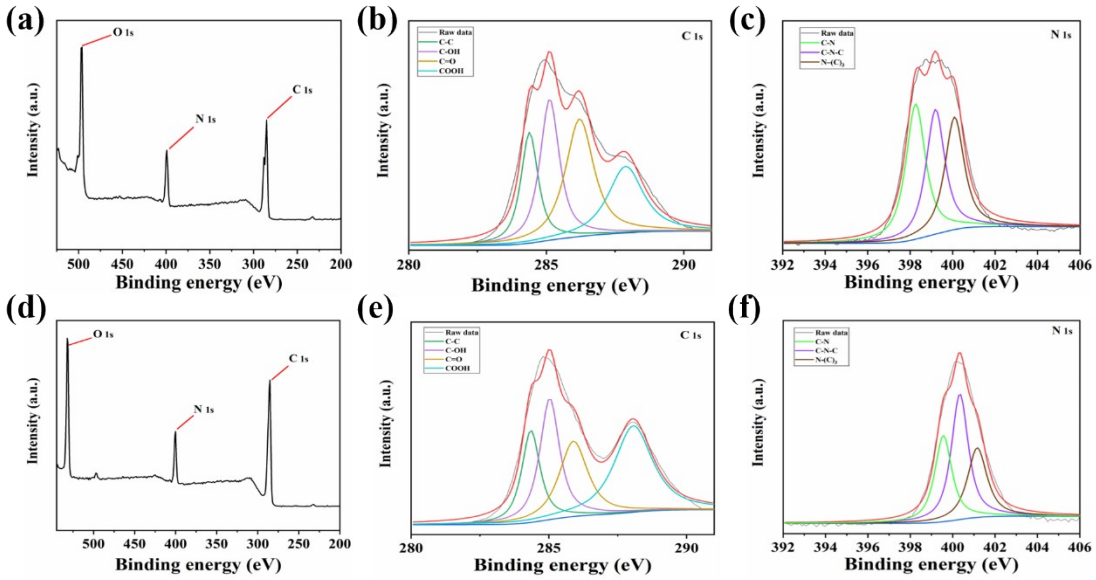

Figure S3. XPS spectra of N-CDs (a, b and c) and PNDs (d, e and f). (a, d) survey spectra; (b, e) high-resolution XPS spectra of C 1s; (c, f) high-resolution XPS spectra of N 1s. The red line represents the fitted curve, and the blue line represents the baseline.

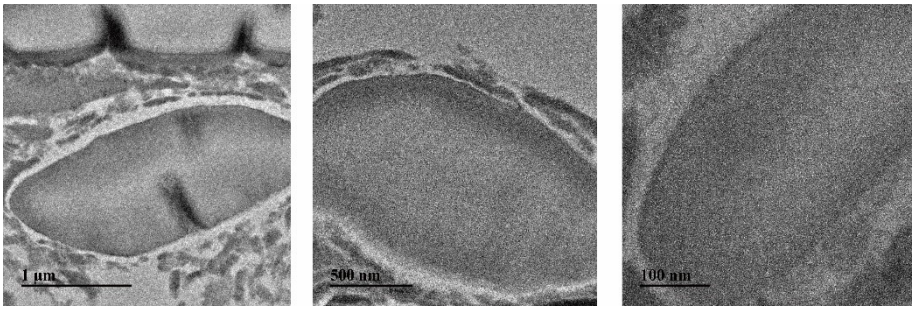

Figure S4. TEM images without PNDs in the chloroplast.

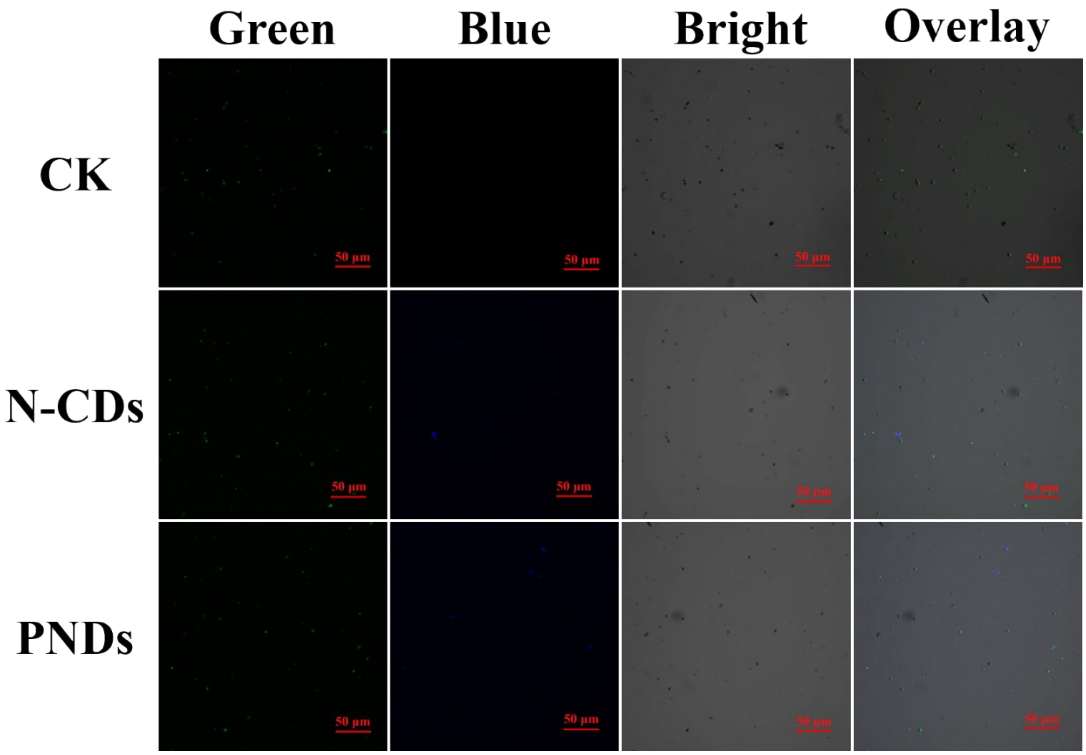

Figure S5. CLSM images of CK, N-CDs and PNDs in chloroplast of maize.

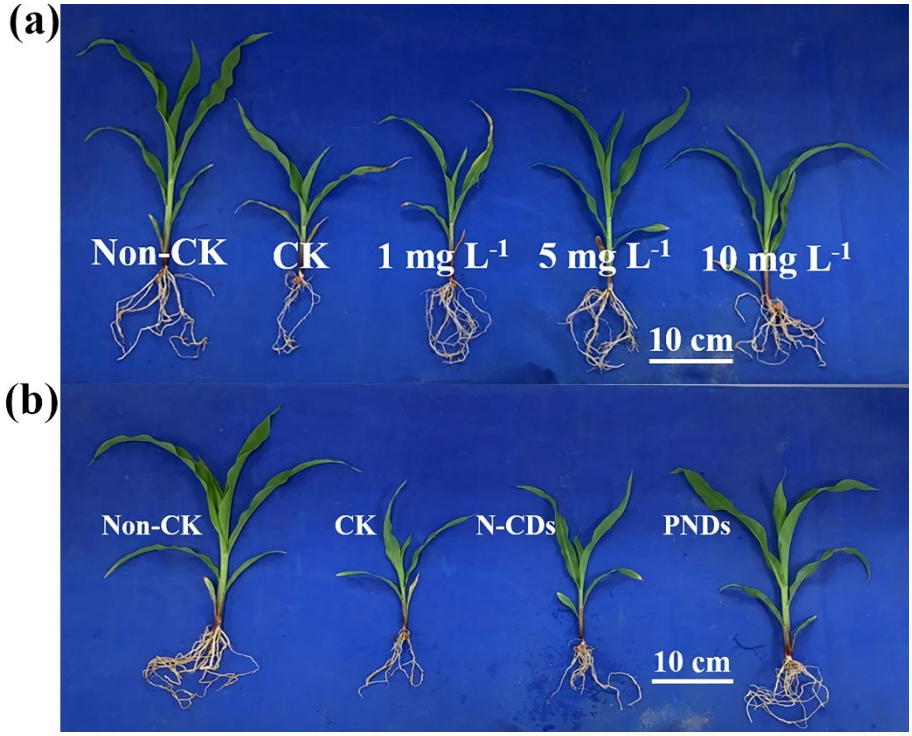

Figure S6. The photo of maize under the various conditions: non-drought (Non-CK), drought (CK), foliar sprayed N-CDs (5 mg·L^{-1}), and PNDs with different concentrations (1, 5, and 10 mg·L^{-1}) under drought.

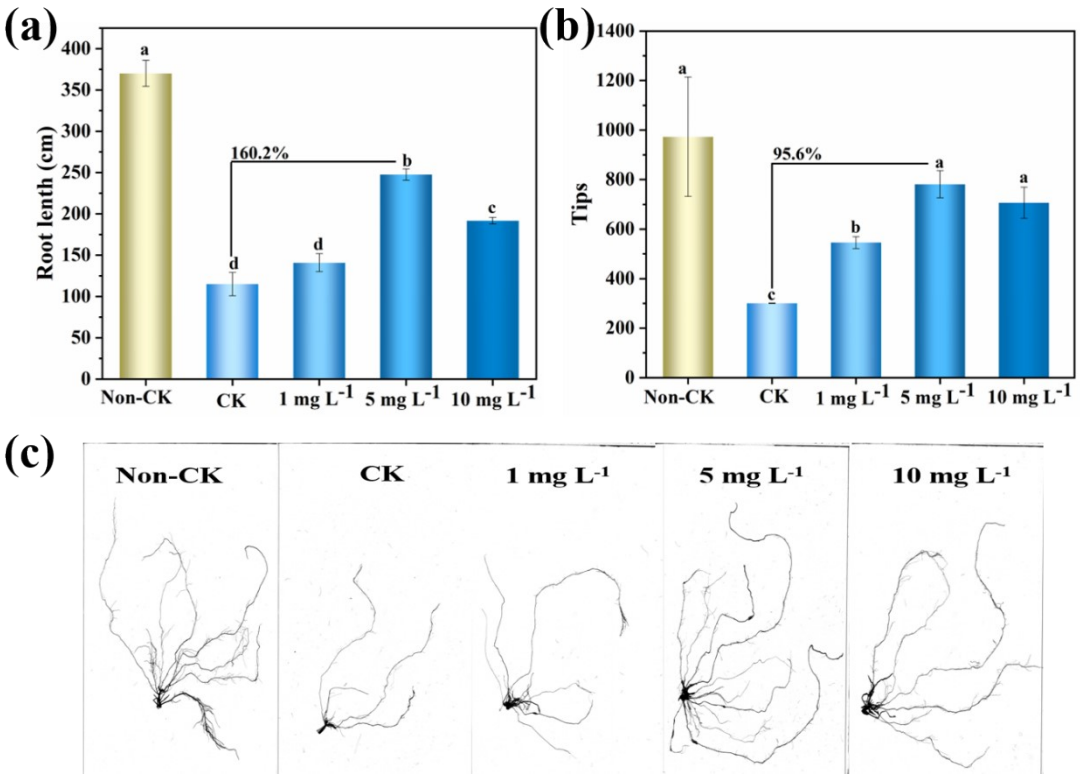

Figure S7. The roots growth of maize. (a) The root length, (b) tips and (c) photographs of maize under non-drought (Non-CK), drought (CK) and foliar sprayed PNDs with different concentrations (1, 5, and 10 mg·L^{-1}) under drought.

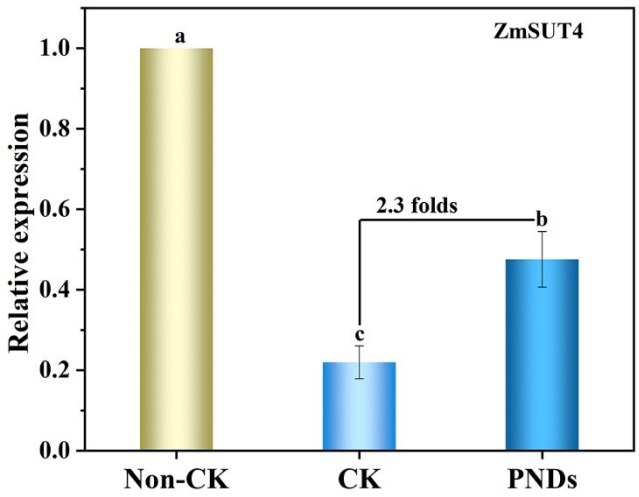

Figure S8. Expression of *ZmSUT4* gene of maize leaf in foliar sprayed PNDs at 5mg·L^{-1} in comparison with the CK and non-CK.

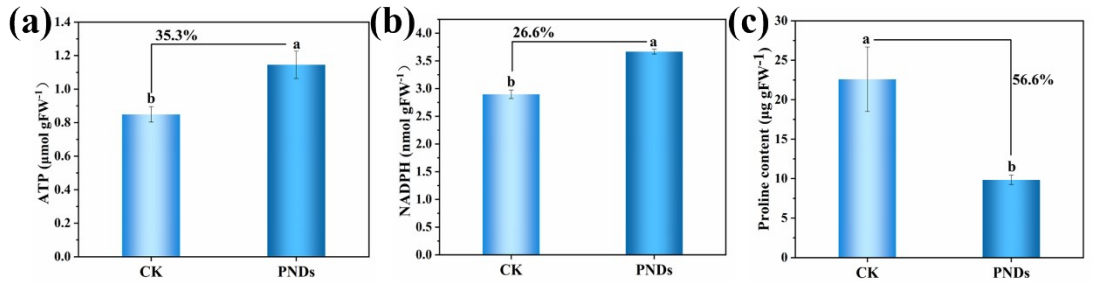

Figure S9. The production of (a) ATP, (b) NADPH and (c) proline content of maize in shoots after foliar sprayed PNDs at 5 mg·L^{-1}.

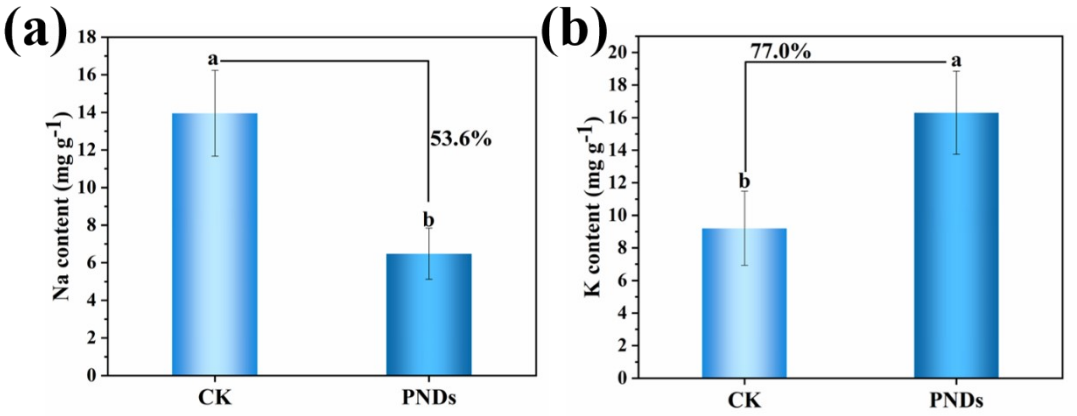

Figure S10. The content of (a) Na^+ and (b) K^+ of corn root after foliar spraying PNDs at 5 mg·L^{-1}.

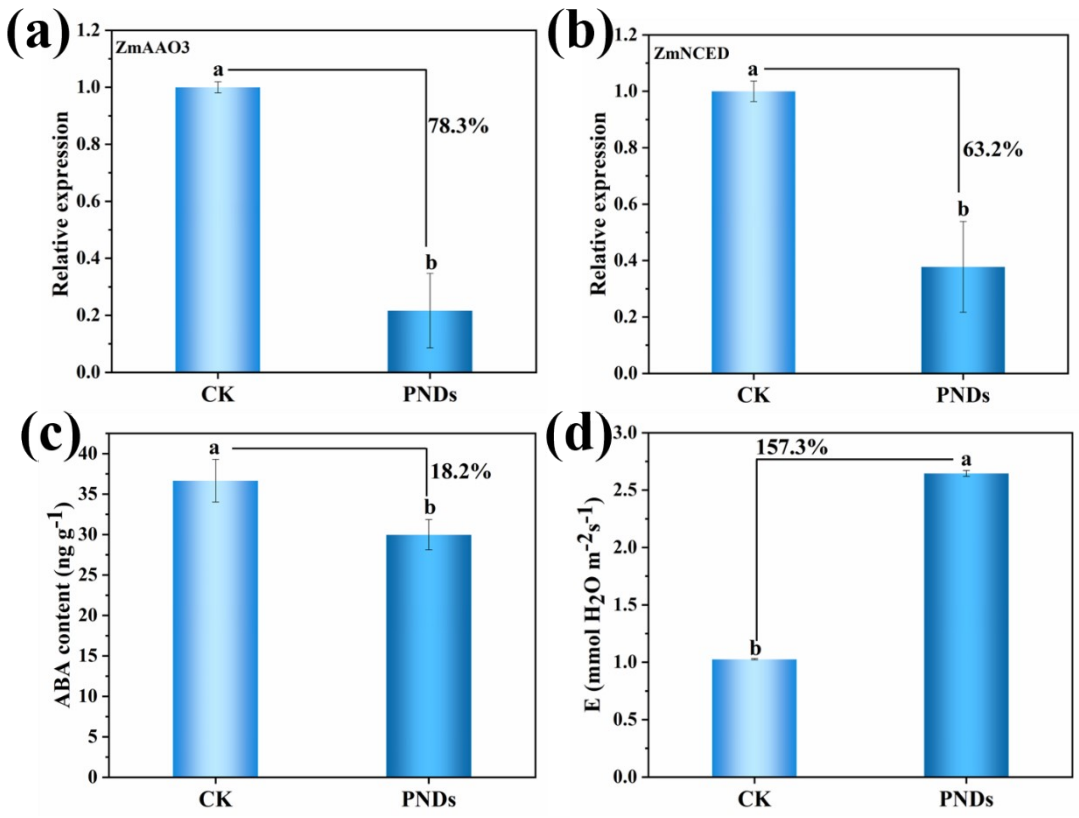

Figure S11. Expression of (a) *ZmAAO3* and (b) *ZmNCED* gene; (c) ABA content and (d) transpiration rate (E) of maize shoots treated with foliar sprayed PNDs at 5 mg·L^{-1}.

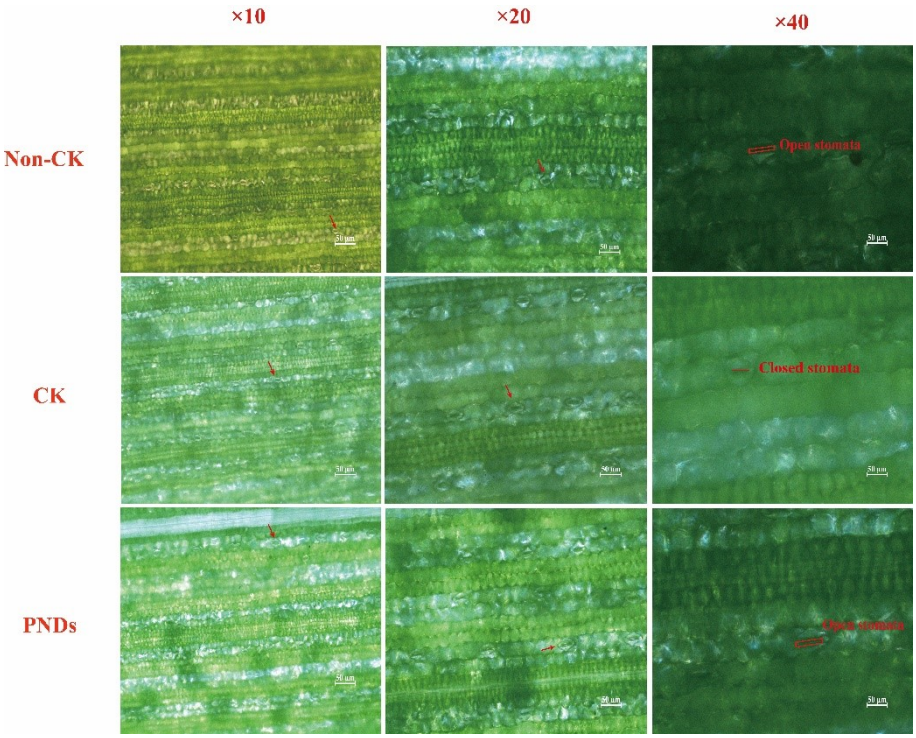

Figure S12. Images of stomatal morphology in Non-CK, CK, and PNDs (5 mg·L^{-1}).

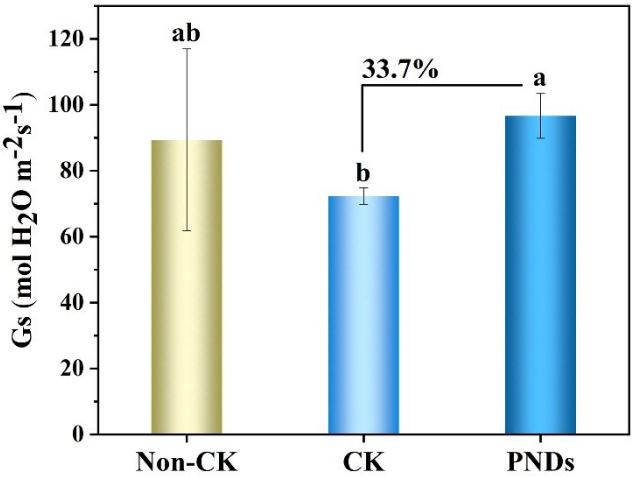

Figure S13. Stomatal conductance of maize leaves Non-CK, CK, and PNDs (5 mg·L^{-1}).

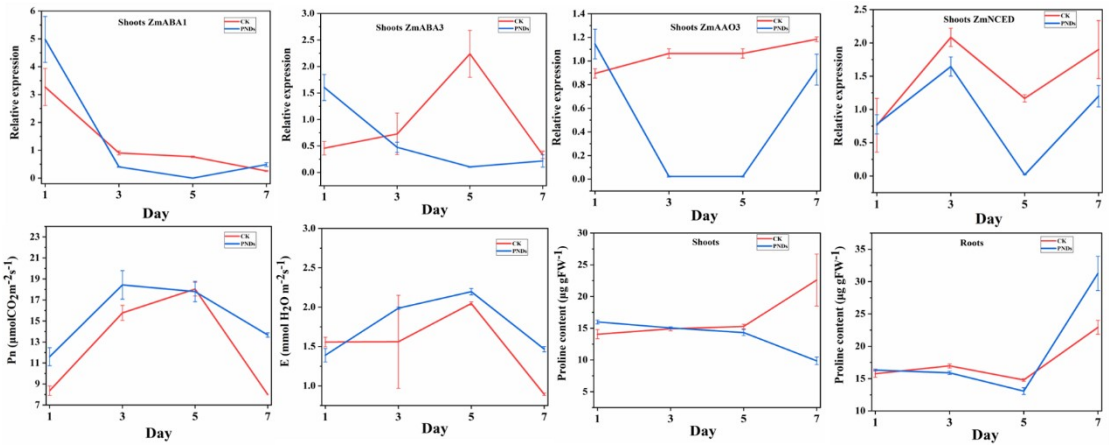

Figure S14. ABA synthesis gene expression (*ZmABA1, ZmABA3, ZmAAO3, ZmNCED*), proline content in leaf; Pn and E in leaf of maize during dynamic process.

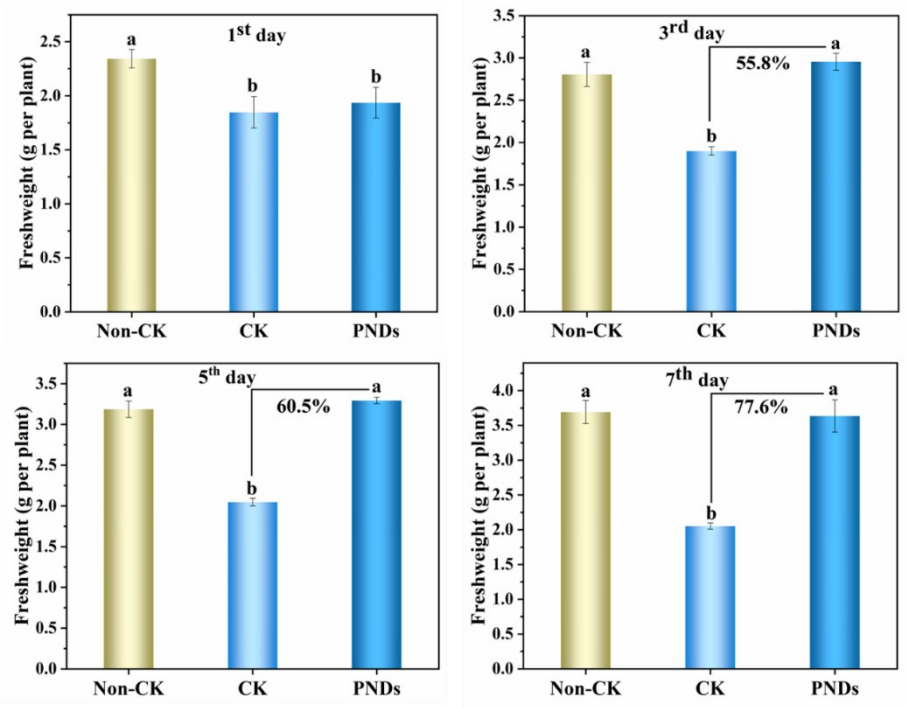

Figure S15. The fresh weight of maize after foliar application of PNDs at 5 mg·L^{-1} during dynamic process.

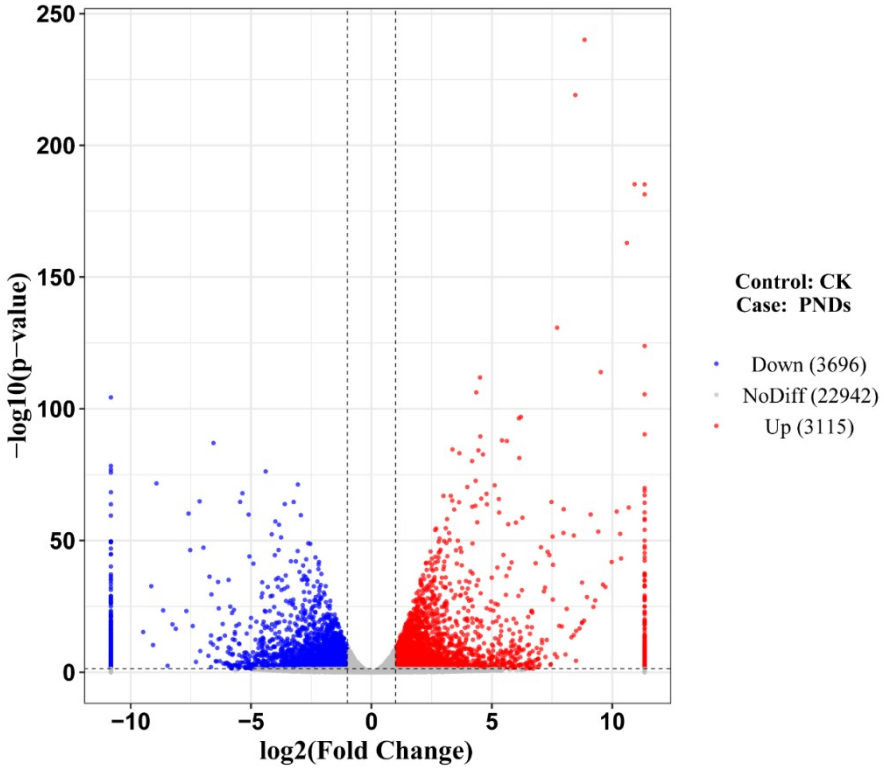

Figure S16. Volcano map of genes after foliar application of PNDs at 5 mg·L^{-1}.

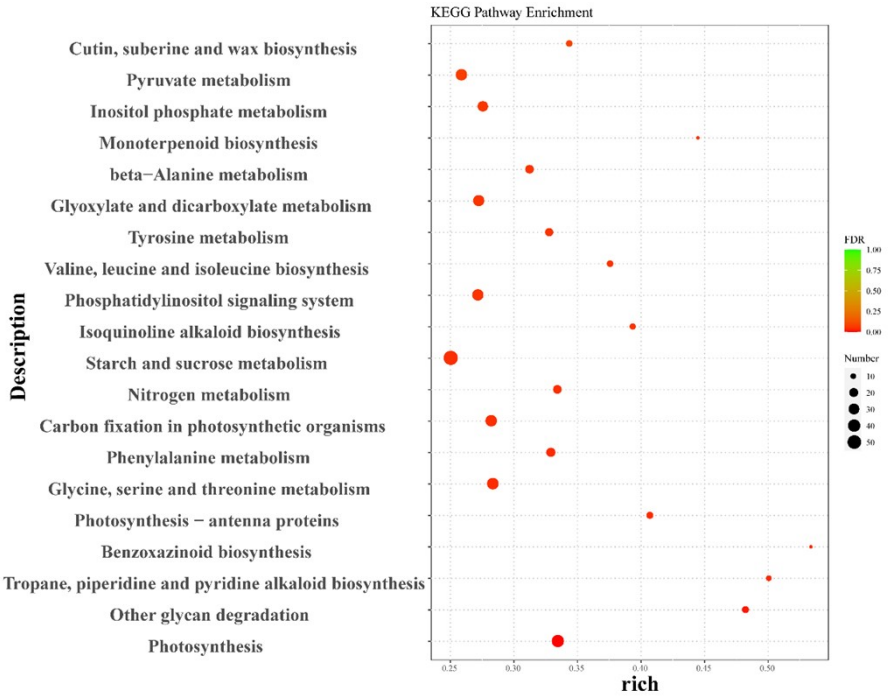

Figure S17. Kyoto Encyclopedia of Genes and Genomes (KEGG) rich distribution point diagram after foliar application of PNDs at 5 mg·L^{-1}.

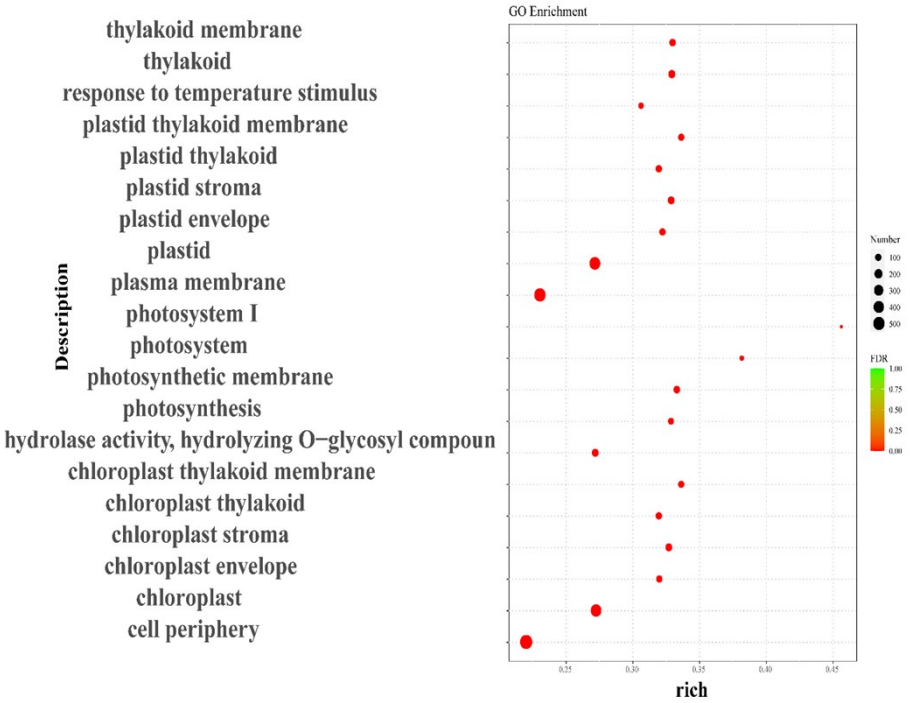

Figure S18. Gene Ontology (GO) enrichment analysis scatter plot after foliar application of PNDs at 5 mg·L^{-1}.

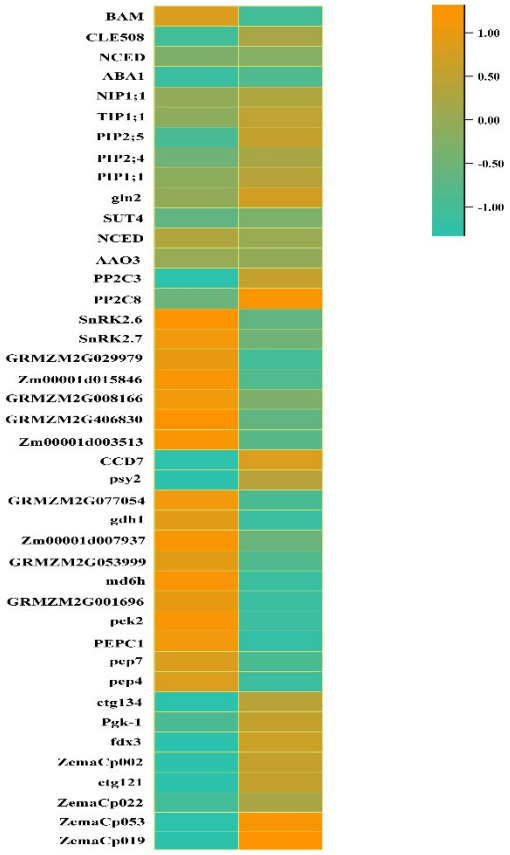

Figure S19. Gene heat map in five signal pathways after foliar application of PNDs at 5 mg·L^{-1}.

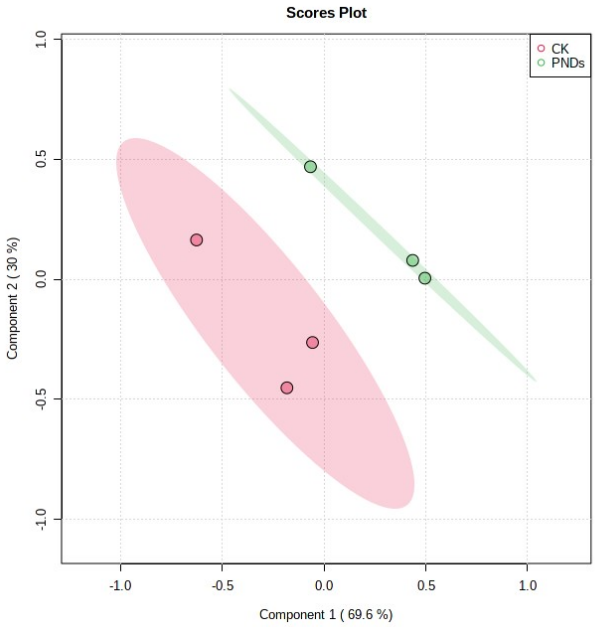

Figure S20. Principal component analysis (PCA) score plots of metabolic profiles in maize shoots treated with PNDs (5 mg·L^{-1}).

References

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