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Journal

Environmental Science and Technology, 59(44)

ISSN

0013-936X

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Publication Date

2025-11-11

DOI

10.1021/acs.est.5c11193

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CuO@SiO₂ Seed Priming Enhances Drought Tolerance and Phosphorus Acquisition Efficiency of Maize

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Cite This: *Environ. Sci. Technol.* 2025, 59, 23837–23848



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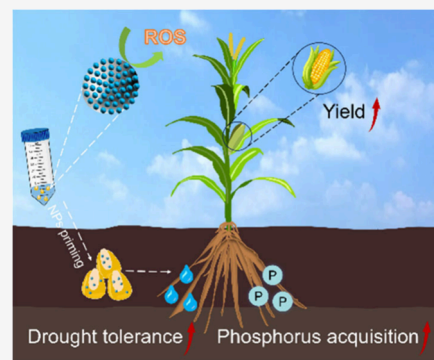
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ABSTRACT: Sustainable agriculture requires minimizing resource inputs while maximizing the outputs. Here, we report that rationally engineered Fenton-like CuO@SiO₂ nanoparticles (NPs), as a seed priming agent (20 mg/L, 24 h), concurrently enhanced drought tolerance and phosphorus (P) acquisition efficiency of maize, a climate vulnerable staple crop. Importantly, life cycle field pot trials reveal that CuO@SiO₂ NPs seed priming increased the grain yield by 9.3% and 16.6%, respectively, under normal and drought conditions, compared to the hydropriming control. This enhanced drought-resilience and yield were attributed to reactive oxygen species (ROS)-intensified root system architecture (RSA) plasticity, including enhanced root hair density, deeper primary root, and prolific lateral root branching. RNA-seq revealed the activation of genes related to RSA modulation (PLA2, PG, and EXP), P/N/S uptake and assimilation (PHT, NRT, NR, NiR, and SULTR), and drought tolerance (ELIP, LEA, and DRP) in CuO@SiO₂ NPs-primed seeds. Additionally, seed priming altered the seed metabolite profile, systematically elevating the content of adenosine triphosphate (24.1%), amino acids (7.6–105%), sugars (20–24%), organic acids (49–121%), phenolic acids (47–315%), and fatty acids (4.2–39%), indicating the boosted respiration and accelerated mobilization of seed stored reserves. Collectively, this simple and inexpensive (\$0.62–1.15 per acre) seed priming strategy effectively enhances maize resilience to water and nutrient stress, offering a promising path toward sustainable food security.

KEYWORDS: climate change, water deficit, phosphorus acquisition, nanoenabled agriculture, omics



INTRODUCTION

Conventional agriculture, which relies heavily on irrigation and agrochemical inputs, is becoming increasingly unsustainable. This is particularly evident in water use: while water is indispensable for plant growth as the primary electron donor in photosynthesis, agriculture consumes 70% of the world's freshwater. This is clearly unsustainable, especially because climate change is projected to worsen water limitations globally.¹ Similarly, phosphorus (P) is an essential macro-nutrient for energy metabolism, DNA/RNA synthesis, and intracellular signaling.^{2,3} However, only 15–30% of applied P fertilizers are actually utilized by crops, with the vast majority becoming unavailable in soil or running off to freshwaters and finally into oceans.⁴ This is already beyond the safe operating space for sustainable human development.⁵ To sustainably support a growing population while safeguarding ecosystems, conventional agriculture will have to undergo a complete transformation: minimizing resource inputs while maximizing outputs.^{6,7} This necessitates innovative approaches to enhance crops' resilience under short-term water scarcity and improve P acquisition and use efficiency without compromising yield.

Root, evolved through the Devonian Period (from 416 to 360 million years ago), serves as the primary organ for water

and nutrient acquisition by terrestrial plant species. Despite their sessile nature, roots exhibit remarkable plasticity through dynamic adjustment of morphology, branching patterns, and growth angles, collectively termed the root system architecture (RSA), to enhance water and nutrient uptake under water-deficit and nutrient-deficient environments.^{8,9} For instance, drought triggers a deeper RSA to acquire subsoil water reserves. Conversely, P deficiency promotes a shallower RSA to maximize P capture from the topsoil layer.¹⁰ Harnessing this adaptive ability, RSA engineering could be a viable strategy to cultivate crops resilient to water-deficit and P-limited conditions.^{9,11} While genetic engineering and synthetic biology are promising to rationally design RSA,^{12,13} progress remains slow, and alternative approaches are needed.

RSA is modulated by both intrinsic phytohormone signaling pathways (e.g., auxin and cytokinin) and exogenous environ-

Received: August 14, 2025

Revised: October 18, 2025

Accepted: October 20, 2025

Published: October 27, 2025



mental conditions, including water stress and nutrient deficiency.¹⁴ Central to this regulatory network are reactive oxygen species (ROS), which function as key signaling hubs that integrate hormonal signaling and stress response pathways.¹⁵ Building on this, we propose a hypothesis that engineered nanoparticles (NPs) with ROS-generating capacities could act as multifunctional seed-priming agents to (1) activate phytohormone pathways and downstream RSA-associated transcriptional regulators, priming roots to dynamically fine-tune architecture under resource-limited conditions and (2) epigenetically precondition seedlings by triggering “stress memory”, enabling faster, stronger responses to subsequent abiotic stresses.

Maize (*Zea mays* L.) is a globally important crop used for food, feed, and biofuel. Maize production has become increasingly threatened as climate change intensifies, largely due to exposure to wider temperature extremes and reduced water availability.¹⁶ Here, we tested whether a rationally designed nanoscale CuO@SiO₂ NPs with ROS-generating capacity can function as seed priming agents to enhance the drought resilience and P acquisition efficiency of maize. This strategy aims to engineer “climate-adaptive RSA” and “climate-resilient” crops. By reducing the necessary inputs of irrigation and phosphate fertilizers while maintaining/increasing yield, this multifunctional approach simultaneously addresses food security and sustainable agriculture. This easy-to-operate seed priming approach offers socioeconomic benefits for small-holder farmers.

MATERIALS AND METHODS

NPs Synthesis and Characterization. CuO@SiO₂ NPs were synthesized via a hydrothermal method.¹⁷ Specifically, SiO₂ NPs (5.0 wt %, Pantian Nano Material Co., Ltd.) were dispersed in a 2.4 g/L aqueous Cu(NO₃)₂ solution. The pH of the mixture was adjusted to 10 using 40 g/L NaOH, followed by stirring for 30 min. The solution was then transferred to a Teflon-lined autoclave reactor and heated at 170 °C for 12 h. The product was collected by centrifugation, washed three times with deionized (DI) water, and then dried at 60 °C for 12 h. Finally, it was calcined at 500 °C for 6 h in an O₂ atmosphere.

The size, morphology, and elemental composition of the CuO@SiO₂ NPs were characterized by transmission electron microscopy (TEM) in conjunction with energy-dispersive X-ray spectroscopy (EDX) using an FEI Talos F200X G2 microscope. The crystal structure was analyzed by X-ray diffraction (XRD) on a Bruker D8 ADVANCE diffractometer with Cu K α radiation ($\lambda = 1.5418$ Å), scanning 2θ from 10° to 80°. The surface elemental states were determined by X-ray photoelectron spectroscopy (XPS) on a PHI 5000 Versaprobe III instrument. The ζ potential of CuO@SiO₂ NPs (20 mg/L in DI water) was measured by dynamic light scattering using a Malvern Instruments Nano-ZS90.

The hydroxyl radical ($\bullet$ OH) generation capability of CuO@SiO₂ NPs was evaluated by electron paramagnetic resonance (EPR) spectroscopy using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) as a spin trap.^{18,19} Briefly, NPs (20 mg/L) were dispersed in an aqueous solution containing DMPO (5.7 g/L) and H₂O₂ (3.4 g/L), and the resulting DMPO/ $\bullet$ OH adduct was detected. The peroxidase (POD)-like activity was assessed by oxidizing 3,3',5,5'-tetramethylbenzidine (TMB). A mixture of CuO@SiO₂ NPs (20 mg/L), H₂O₂ (1.1 g/L), and TMB (48.1 mg/L) in a sodium acetate buffer (3 mL, pH 5.0) was

incubated at 37 °C for 60 min. The formation of oxidized TMB (oxTMB) was quantified by measuring the absorbance at 650 nm using a Varioskan LUX microplate reader (Thermo Scientific).

Experimental Design of Drought-Tolerance Assay.

Germination Stage. Maize (*Zea mays* L.) seeds were purchased from Agria NKY Seed Co., Ltd. (Beijing, China). Seeds were surface-sterilized with 5% NaClO for 10 min and rinsed thoroughly with DI water. For priming, seeds were immersed in either a CuO@SiO₂ NPs suspension at 20 mg/L (for screened from preliminary experiments, see [Supplementary Figure S1](#)) or DI water (hydropriming) for 24 h at 25 °C in the dark with continuous shaking (100 rpm). The primed seeds were placed in Petri dishes (20 seeds/dish) and moistened with 3 mL of either DI water for normal conditions or a 15% poly(ethylene glycol) (PEG) solution to simulate drought. For each treatment, four replicate dishes were incubated at 25 °C in the dark (70% relative humidity) for 7 days. Germination, defined by radicle protrusion exceeding 50% of the seed length, was recorded at 3 h intervals. The vigor index was calculated according to the method of Abbasi Khalaki et al.²⁰ (details are given in [Supplementary Text S1](#)).

Vegetative Stage. To test whether seed priming could increase drought tolerance of maize vegetative tissues, water- or CuO@SiO₂ NPs-primed seeds were directly sown in plastic pots (the height and upper and lower diameters were 12 cm $\times$ 9 cm $\times$ 6 cm) containing 50 g of artificial soil [80% nutrient soil (Scotts Miracle-Gro Co., Marysville, OH), 10% vermiculite, and 10% sand] and cultivated in a greenhouse at 28 °C on a 16 h photoperiod. Drought treatment was imposed on 2-week-old maize plants by stopping irrigation for 2 weeks. At day 28, maize seedlings were harvested. Plant height, shoot fresh weight, root length, biomass, and leaf relative water content (RWC) were measured. For malondialdehyde (MDA) and photosynthetic pigments analysis, fresh leaf tissues were ground into powder using a mortar and pestle in liquid nitrogen and stored at −80 °C until use.

Life Cycle Pot Experiment. For life cycle study, maize seeds primed with water or CuO@SiO₂ NPs were sown in plastic pots (the height and upper and lower diameters were 34 cm $\times$ 32 cm $\times$ 26 cm) containing 7 kg of a growth medium [a mixture of local real soil and potting soil (Scotts Miracle-Gro Co., Marysville, OH) at a ratio of 1:1] and allowed to grow outdoors at Nanjing University campus for 3 months (July–October, 2024). Each treatment contained eight replicate pots, with one plant per pot. The maize plants were irrigated two times per week. For drought treatment, irrigation was stopped for 1 week at the three-leaf stage, jointing stage, and tasseling stage of corn development, a total of three times. Commercial fertilizer (N–P₂O₅–K₂O $\geq$ 45%, 15:15:15) was applied one time per month and at 10 g/pot. There was no pesticide spraying. At harvest, yield-related parameters, including the shoot length, ear of corn length, and grain dry weight (g/100 grains), were measured.

Maize P Acquisition Efficiency Assay. To test whether seed priming could enhance the P and nitrogen (N) acquisition, primed seeds were sown in plastic pots (12 cm $\times$ 9 cm $\times$ 6 cm) containing 150 g of local real soil and cultivated in a greenhouse at 28 °C on a 16 h photoperiod. A total of 2 g of sparingly soluble P fertilizer (Ca₁₀(PO₄)₆F₂) or readily soluble P fertilizer (Ca(H₂PO₄)₂) was added per plant to simulate Low-P and High-P conditions. At day 40, maize

seedlings were harvested, and the shoot length and fresh biomass, root length, and P and N contents were measured.

MDA and Photosynthetic Pigments Measurements. The MDA content was measured by the Thiobarbituric Acid Reactive Substances (TBARS) assay,²¹ and the photosynthetic pigments (chlorophyll a and b and carotenoid) were determined using a BioReader (Varioskan LUX, Thermo Scientific, Vantaa, Finland), following extraction with a 1:1 (v/v) mixture of 80% acetone and 100% ethanol (details are given in [Supplementary Text S2](#)).

Adenosine Triphosphate (ATP) Content in Primed Seeds. The ATP concentration in primed seeds was quantified using a commercial ATP Assay Kit (Beyotime, Cat. No. S0026B). Briefly, seeds primed with water or CuO@SiO₂ NPs for 24 h were thoroughly washed, ground in liquid nitrogen, and powdered. A 0.1 g portion of this powder was homogenized in 0.9 mL of lysis buffer for 5–10 min, heated at 100 °C for 2 min, and then cooled on ice. The homogenate was centrifuged at 10000g for 15 min at 4 °C. A 20 μ L portion of the resulting supernatant was transferred to a luminometer plate. After the addition of 100 μ L of an ATP detection working solution, luminescence was measured immediately on a microplate reader (Maxwell Technologies, San Diego, CA). Sample ATP concentrations were determined against a standard curve, and all measurements were performed in triplicate.

ROS Levels in Primed Seeds. The ROS levels of primed maize seeds were visualized and quantified by fluorescent microscopy with 2',7'-dichlorofluorescein diacetate (DCFH-DA) as a fluorescent probe.²² Briefly, primed maize seeds were embedded in 10 μ M DCFH-DA in phosphate-buffered saline (PBS; pH 7.2) for 30 min at 37 °C in darkness. The maize seeds were then washed three times in the same buffer for 5 min. DCFH-DA green fluorescence (excitation at 488 nm and emission at 525 nm) was observed and photographed with fluorescence microscopy (Olympus BX53 U-RFL-T, Tokyo, Japan). The fluorescence intensity of ROS levels was measured by *ImageJ* software (ImageJ 1.54g, NIH, Bethesda, MD).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis. The copper (Cu) content in primed seeds was quantified by ICP-MS (NexION-300, PerkinElmer, Shelton, CT). Specifically, water- or CuO@SiO₂ NPs-primed seeds were thoroughly washed with DI water. Then, the seed coat, embryo, and endosperm were carefully dissected. The isolated tissues were oven-dried at 75 °C for 48 h. Approximately 100 mg of each dried tissue sample was digested with 5 mL of a 1:4 (v/v) mixture of plasma-pure HNO₃ and H₂O₂ using a microwave digestion system (Milestone Ethos Up, Bergamo, Italy) at 160 °C for 120 min. After cooling, the digestion solution was diluted, filtered, and analyzed by ICP-MS.

P and Nitrate Contents in Maize Tissues. P was quantified by measuring the absorption at 485 nm using a BioReader (Varioskan LUX, Thermo Scientific, Vantaa, Finland).²³ Shoot and root dry tissues were digested as described above; 10 mL of digesta was then diluted with 25 mL of DI water, followed by the addition of 5 mL of HNO₃, 5 mL of ammonium molybdate (5%), and 5 mL of ammonium metavanadate (0.25%).

The nitrate concentration was measured as described.²⁴ Briefly, 0.2 g of leaf dry tissues was boiled in 5 mL of DI water for 30 min. A total of 3 mL of supernatant was then filtered through a polyether sulfone ultrafiltration membrane with a pore size of 0.22 μ m, and 0.1 mL of the filtrate was mixed with

0.4 mL of a salicylic acid–sulfuric acid solution, followed by incubation for 20 min at ambient temperature. Then 9.5 mL of 8% (w/v) NaOH was added to stop the reaction; the nitrate concentration was determined with a microplate reader at 410 nm.

RNA-seq Analysis. After priming for 24 h with CuO@SiO₂ NPs (20 mg/L) or water, the seed samples were collected for transcriptome analysis. Total RNA was extracted from the tissue using TRIzol Reagent according to the manufacturer's instructions (Invitrogen), and genomic DNA was removed using DNase I (Takara). The RNA quality was determined using a 2100 Bioanalyser (Agilent) and quantified using a ND-2000 (NanoDrop Technologies). High-quality RNA samples (OD_{260/280} = 1.8–2.2, OD_{260/230} $\geq$ 2.0, RIN $\geq$ 6.5, 28S:18S $\geq$ 1.0 and $> 10 \mu$ g) were used to construct the sequencing library. Four biological replicates for each treatment were sequenced. Paired-end libraries were sequenced by Illumina NovaSeq 6000 sequencing (150bp*2, Shanghai BIOZERON Co., Ltd., Shanghai, China). The expression level for each gene was calculated using the fragments per kilobase of exon per million mapped reads (FRKM) method to identify differentially expressed genes (DEGs, with FDR $\leq$ 0.05 and $\log_2$ FCI $\geq$ 1) between the different samples. The R statistical package edgeR (Empirical Analysis of Digital Gene Expression in R, <http://www.bioconductor.org/packages/release/bioc/html/edgeR.html>) was used for differential expression analysis. To understand the functions of the DEGs, gene ontology (GO) functional enrichment and KEGG pathway analysis were carried out by Goatoools (<https://github.com/tanghaibao/Goatoools>) and KOBAS (<http://bioinfo.org/kobas3/>). DEGs were significantly enriched in GO terms and metabolic pathways when their Bonferroni-corrected *p* value was less than 0.05.

Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS) Analysis of Metabolites in Maize Seeds. Metabolites in seeds were quantified by LC–MS/MS. Specifically, seeds primed with water or CuO@SiO₂ NPs for 24 h were washed three times with nanopure water to remove residual priming agents. The washed seeds were then flash-frozen in liquid nitrogen, pulverized into fine powder using a prechilled mortar and pestle, and stored at –80 °C pending subsequent analysis. For amino acids analysis, 100 mg of powders were extracted with 1 mL of 0.5 mol/L HCl, and 250 μ L of the supernatant was collected and transferred to LC–MS vials with 750 μ L of 100% acetonitrile. For sugar analysis, the extraction solution was replaced with 1 mL of 50% methanol. In the case of acid metabolite analysis, 1 mL of methanol was used as an extraction solution. More detailed protocols were described in previous studies.²⁵ Qualitative analysis of extracts was performed on a high-performance liquid chromatography (HPLC) system (Agilent 1260) coupled to an electrospray ionization triple-quadrupole mass spectrometer (AB SCIEX API4000). Instrument control, data acquisition, and processing were performed using *MultiQuant* software, version 2.1 (SCIEX). A supervised sparse partial-least-squares discriminant analysis (sPLS-DA) clustering method was run on the metabolomics data set using *MetaboAnalyst 5.0* (<http://www.metaboanalyst.ca/>).²⁶

Statistical Analysis. Data were processed using Microsoft Excel 2021 and GraphPad Prism 8, and a Student's *t* test was performed to evaluate the significance of the difference between treatments. For all comparisons, *p* < 0.05 was considered statistically significant. Diagram plotting was

carried out using GraphPad Prism 8 and Adobe Illustrator 2020.

RESULTS AND DISCUSSION

Design, Synthesis, and Characterization of CuO@SiO₂ NPs. We rationally designed and synthesized core–shell CuO@SiO₂ NPs, in which a CuO shell encapsulates a SiO₂ NPs core (Figure 1A). The rationale for this core–shell design

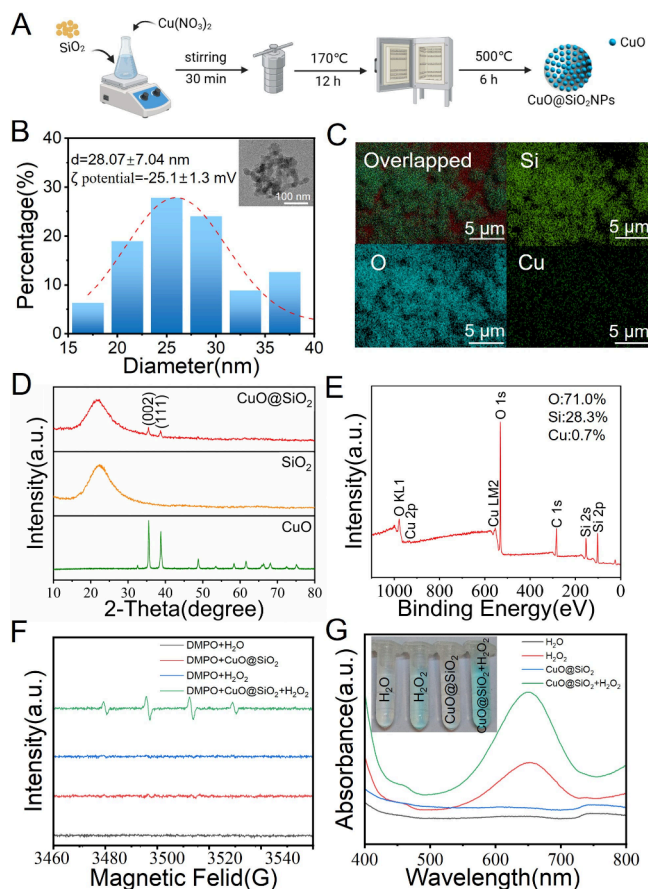


Figure 1. Characterization of CuO@SiO₂ NPs. (A) Synthesis steps of CuO@SiO₂ NPs. (B) TEM image, size distribution, and ζ potential. (C) Scanning electron microscopy with EDX. (D) Powder XRD pattern of the synthesized CuO@SiO₂ NPs. (E) Full-scale XPS spectra of CuO@SiO₂ NPs. (F) EPR results. (G) UV–vis absorption spectra of the TMB/H₂O₂ system. Inset: Photograph of colored oxTMB solutions with CuO@SiO₂ NPs + H₂O₂, CuO@SiO₂ NPs, H₂O₂, and H₂O.

is based on three key factors: (1) overcoming the inherent instability of CuO NPs; (2) ensuring high Cu utilization efficiency by exposing only a small amount of active Cu on the surface; (3) leveraging a potential synergistic effect because the fumed silica core not only provides a rigid scaffold but also possesses intrinsic ROS-generating capability. This design rationale is strongly supported by our preliminary data, which demonstrate that the CuO@SiO₂ core–shell structure outperforms the individual components (CuO NPs or SiO₂ NPs alone) in enhancing maize seed germination speed, shoot and root length, and seedling vigor (Supplementary Figure S2). TEM revealed spherical morphology with an average diameter of 28 ± 7 nm (Figure 1B). EDX analysis confirmed the presence of Cu, silicon (Si), and oxygen (O) (Figure 1C). The

ζ potential of CuO@SiO₂ NPs was -25.01 ± 1.3 mV, suggesting good colloidal stability in an aqueous suspension. XRD patterns confirmed the crystalline nature of CuO, showing characteristic peaks at 35.5° and 38.7° that correspond to the (002) and (111) planes, respectively (Figure 1D). XPS further verified the material composition, indicating atomic ratios of O (71.0%) and Si (Figure 1E). EPR analysis revealed a distinct 1:2:2:1 quartet signal in the presence of H₂O₂, confirming the $\bullet$ OH-generation capability of CuO@SiO₂ NPs (Figure 1F). Furthermore, TMB colorimetric assays validated the POD-like activity of CuO@SiO₂ NPs (Figure 1G). Collectively, these results demonstrate the Fenton-like properties of the CuO@SiO₂ NPs.

Enhanced Drought Tolerance at the Germination Stage. We first evaluated the performance of CuO@SiO₂ NPs seed priming on germination under PEG-induced drought stress. Seed germination and seedling growth were significantly suppressed under drought exposure, as evidenced by delayed germination speed, reduced seedling vigor, decreased shoot and root lengths, and decreased dry biomass (Figure 2A). Conversely, seeds primed with CuO@SiO₂ NPs exhibited notable drought tolerance, characterized by accelerated germination speed, significantly ($p < 0.05$) enhanced seedling vigor (24%), longer roots and shoots (21% and 30%, respectively), and increased dry weight (34.5%) compared to hydroprimed seeds (Figure 2B–D and Supplementary Figure S3).

Root hairs play a critical role in the acquisition of both water and P, particularly under drought and Low-P conditions.²⁷ Under drought stress, seedlings grown from CuO@SiO₂ NPs-primed seeds exhibited significantly ($p < 0.05$) longer root hairs (24.9%) and greater density (24.3%) compared to those from hydroprimed seeds (Figure 2E–G). Long and dense root hairs enhance the plant's capacity to capture water and nutrients from soil,²⁸ likely contributing to the observed enhanced drought tolerance with nanoscale treatment.

Enhanced Drought Tolerance in Vegetative Tissues. To determine if CuO@SiO₂ NPs seed priming enhances drought tolerance in vegetative tissues, 15-day-old maize seedlings from primed seeds were subjected to a 14-day irrigation withdrawal to impose mild drought stress. Drought stress significantly ($p < 0.05$) reduced the shoot length and shoot fresh weight by 29% and 72.9%, respectively (Figure 3A–C). However, CuO@SiO₂ NPs-primed seedlings exhibited a 9.4% greater shoot length and 55.7% higher shoot biomass compared to hydroprimed seedlings under drought stress (Figure 3A–C). Notably, the leaf RWC was significantly ($p < 0.05$) increased by 3.3% in CuO@SiO₂ NPs-primed seedlings compared to hydroprimed control under drought conditions (Figure 3D).

Drought induces oxidative stress and ROS overaccumulation, resulting in membrane and organelle damage.²⁹ We found that drought stress significantly ($p < 0.05$) increased the MDA content, a biomarker of membrane damage, by 165.8% in leaves compared to those grown under nonstressed conditions (Figure 3E). Conversely, seed priming with CuO@SiO₂ NPs reduced drought-induced MDA accumulation by 17.9% compared to hydroprimed controls under a water deficit (Figure 3E), demonstrating the mitigation of lipid peroxidation. In addition, drought stress resulted in significantly ($p < 0.05$) decreased root length and root biomass in hydroprimed seedlings (Figure 3G–I). Conversely, CuO@SiO₂ NPs primed seedlings exhibited a significant ($p < 0.05$) increase in root

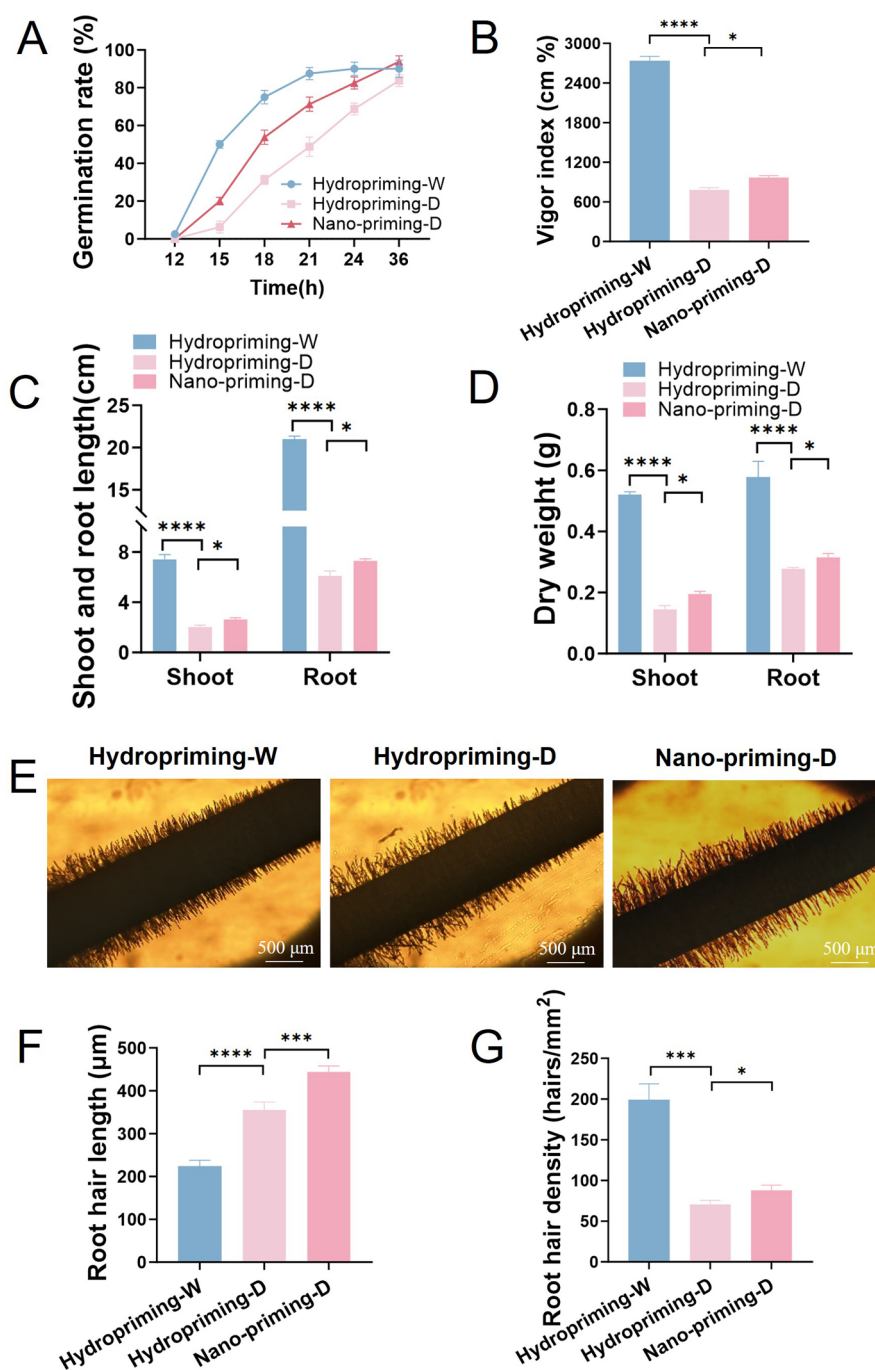


Figure 2. Effects of CuO@SiO₂ NPs seed priming on maize germination speed (A), seedling vigor (B), and shoot and root biomass (C and D) under drought conditions growth for 7 days. (E–G) Root hair length and density. Abbreviations: W, well watered; D, 15% PEG-simulated drought stress. Data are mean $\pm$ standard deviation (each treatment had four replicated samples; there were 20 seeds in each replicate). * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001 as determined by a two-tailed Student's t test.

length (15.2%) and root dry weight (25.2%) relative to hydroprimed seedlings under drought conditions (Figure 3G–I), reflecting the enhanced RSA, which likely improved the root water uptake efficiency and contributed to the observed higher leaf RWC.

Notably, under normal conditions, CuO@SiO₂ NPs-primed seedlings exhibited significantly (p < 0.05) higher shoot and root biomass, increased shoot and root length, and elevated shoot chlorophyll content compared to hydroprimed controls (Figure 3A–I). These results demonstrate that CuO@

SiO₂NPs seed priming improved the photosynthetic efficiency and vegetative growth performance.

Full Life Cycle: Increased Yield under Normal and Drought Conditions. To assess whether CuO@SiO₂NPs priming confers sustained drought tolerance across the entire plant life cycle, a life cycle pot experiment was conducted during the 2024 growing season (July–October). Notably, hydroprimed control plants displayed suboptimal growth under nonstressed conditions, characterized by shorter cob lengths (Figure 3J), likely due to extreme heatwaves that occurred during the summer of 2024 (detailed historical

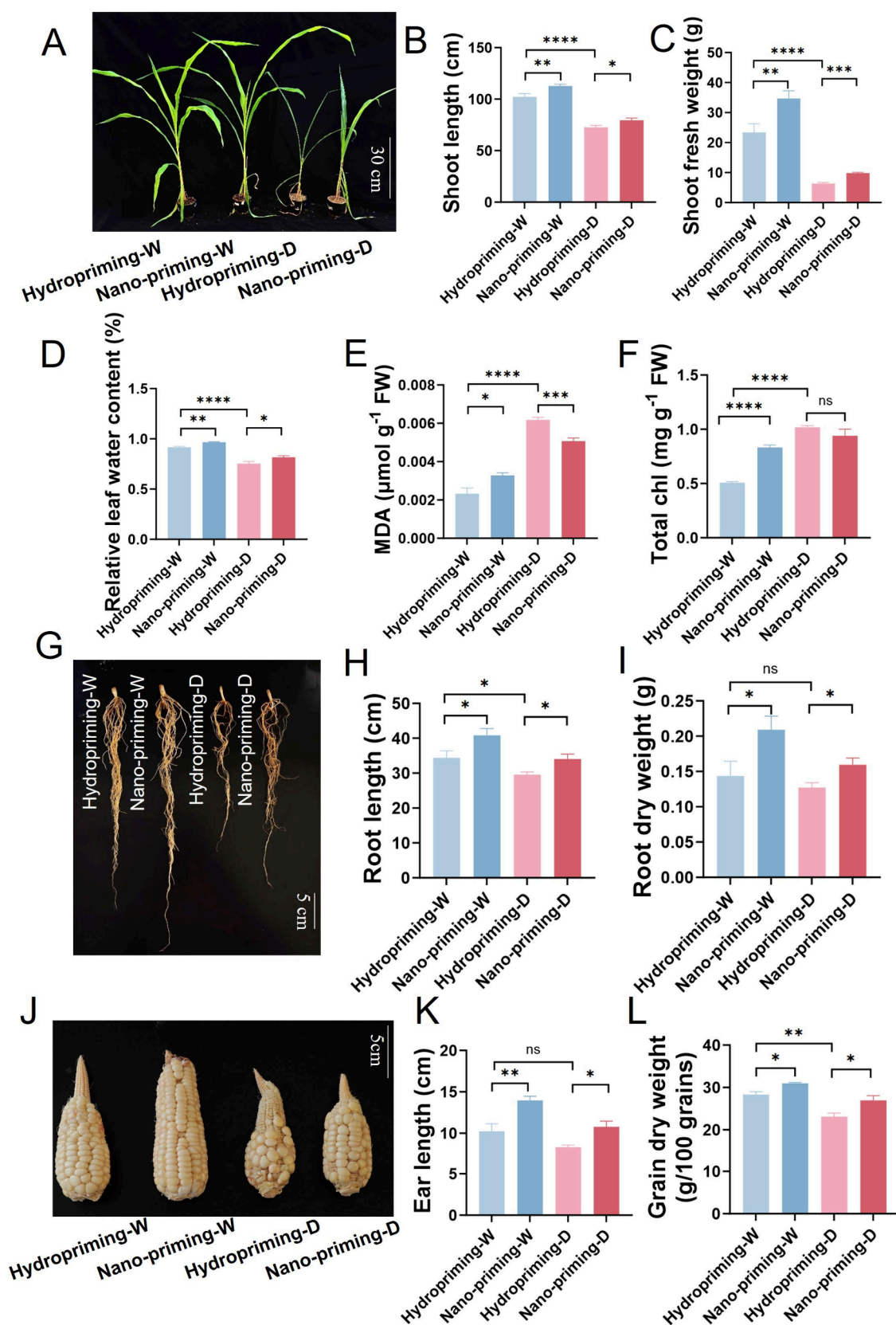


Figure 3. Effects of CuO@SiO₂ NPs seed priming on seedling and whole life cycle growth. (A) Seedling image after 4 weeks of growth. (B–D) Shoot length, fresh weight, and leaf RWC. (E and F) MDA and chlorophyll contents of fresh leaves. (G) Root image after 4 weeks of growth. (H and I) Root length and dry weight. (J) Maize ear image after 3 months of growth. (K and L) Ear length and grain dry weight. Abbreviations: W, well watered; D, drought stress. Data are mean $\pm$ standard deviation ($n = 8$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ as determined by a two-tailed Student's t test.

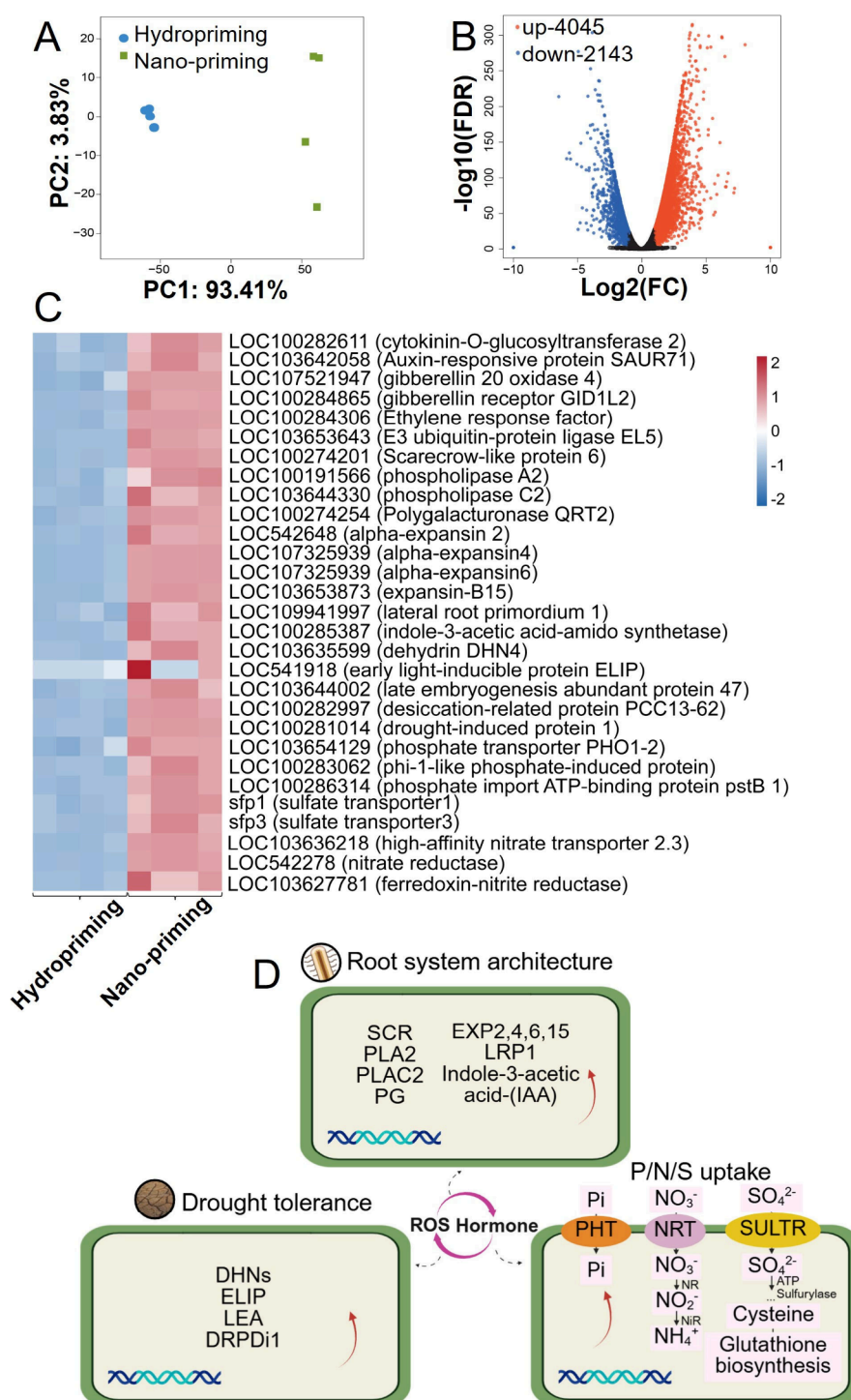


Figure 4. CuO@SiO₂ NPs seed priming reprograms gene expression. (A) PCA of the RNA-seq data obtained from maize seed CuO@SiO₂ NPs primed for 24 h. (B) Volcano plots showing upregulated and downregulated genes ($n = 4$). Threshold for screening DEGs: $|\log \text{FC}| \geq 1$ and $\text{FDR} \leq 0.05$. (C) Heatmap showing the expression of hormones-signaling-related, RSA-related, drought-responsive-related, and P/N/S-uptake-related genes. (D) Schematic diagram of the genes related to drought tolerance, RSA, P/N/S uptake, and assimilation. The figure was created using BioRender.

weather data are listed in [Supplementary Table S1](#)). In contrast, CuO@SiO₂ NPs priming significantly mitigated this unplanned heat-stress-induced growth inhibition. Under drought, CuO@SiO₂ NPs-primed plants exhibited significant ($p < 0.05$) increases in shoot length (11.6%) ([Supplementary Figure S4](#)), ear length (36.6%), and grain dry weight (16.6%) relative to hydroprimed controls ($p < 0.05$; [Figure 3K,L](#)).

Remarkably, CuO@SiO₂ NPs-primed plants under drought stress achieved yields comparable to hydroprimed plants grown under nonstressed conditions ([Figure 3L](#)), demonstrating the potential of this strategy to maintain maize productivity under adverse environmental conditions.

Notably, under nonstressed conditions, CuO@SiO₂ NPs priming also significantly ($p < 0.05$) increased shoot length

(9.2%) (Supplementary Figure S4), ear length (33.6%), and grain dry weight (9.3%) compared to hydroprimed controls (Figure 3K,L). Together, these findings demonstrate that CuO@SiO₂ NPs priming overcomes the growth-defense trade-off, simultaneously enhancing resilience to abiotic stress (drought) and boosting yield under both optimal and stressed conditions. Notably, the enhanced growth in the full life cycle study was unintentionally a dual stress exposure (both drought and heat), but nanoscale seed priming still significantly promoted plant performance.

Improved P Acquisition Efficiency. To evaluate whether CuO@SiO₂ NPs seed priming could increase the P uptake efficiency, a greenhouse pot experiment was conducted using P-deficient soil supplemented with sparingly soluble P fertilizer (Ca₁₀(PO₄)₆F₂) or readily soluble P fertilizer (Ca(H₂PO₄)₂), establishing three treatments: No-P, Low-P, and High-P. After 40 days of cultivation, CuO@SiO₂ NPs-primed seedlings exhibited greater shoot length and shoot fresh weight compared to hydroprimed controls across all P conditions: by 25.3% and 71.2% ($p < 0.05$) in No-P, 4.3% ($p = 0.27$) and 15.4% ($p = 0.15$) in Low-P, and 11.6% and 27.1% ($p < 0.05$) in High-P treatments (Supplementary Figure S5A–C).

As shown in Supplementary Figure S5D,E, the primary root dry weight in CuO@SiO₂ NPs-primed seedlings was significantly ($p < 0.05$) increased compared to hydroprimed controls under the No-P (130%), Low-P (35.9%), and High-P (42.1%) groups. Importantly, the root length was significantly ($p < 0.05$) decreased by 19.5% upon CuO@SiO₂ NPs priming under H–P (Supplementary Figure S5F). The lateral root (LR) number and length are additional dominant features of RSA.³⁰ Under High-P, the LR number in CuO@SiO₂ NP-primed seedlings significantly ($p < 0.05$) increased by 24.4% compared to hydroprimed seedlings (Supplementary Figure S5G). Under No-P and Low-P conditions, the LR number nonsignificantly increased by 6.7% ($p = 0.34$) and 4.9% ($p = 0.29$), respectively. Collectively, these results demonstrate that CuO@SiO₂ NPs priming intensifies RSA plasticity by enhancing both primary and LR development, although the magnitude may be impacted by the P content in the soil.

Given that greater LR branching enables roots to acquire more P from the topsoil, the P content in maize roots and leaves was quantified. Interestingly, the P content in CuO@SiO₂ NPs-primed seedlings (roots + leaves) was significantly ($p < 0.05$) greater than that in hydroprimed controls under No–P (8.0%), Low-P (8.4%) and High-P (8.6%) (Supplementary Figure S5H), demonstrating enhanced P acquisition efficiency from soil.³¹ Notably, the leaf nitrate content also increased significantly ($p < 0.05$) with nanoscale seed priming: by 22.1% (No-P), 27.1% (Low-P), and 18.9% (High-P) (Supplementary Figure S5I). This suggests that CuO@SiO₂ NPs seed priming simultaneously increased the acquisition efficiency of both P and N in maize.

CuO@SiO₂ NPs-Triggered ROS Play a Key Role. We hypothesize that the mechanism for the enhanced drought tolerance and P acquisition is driven by CuO@SiO₂ NPs-induced ROS. To investigate this, we first tested whether CuO@SiO₂ NPs entered the seeds during priming. ICP-MS analysis revealed significantly ($p < 0.05$) greater Cu content in the seed coat and embryo of CuO@SiO₂ NP-primed seeds compared to hydroprimed controls (Supplementary Figure S6), indicating internalization of CuO@SiO₂ NPs during seed priming.

Next, *in vivo* ROS generation in seeds during priming was evaluated. CuO@SiO₂ NPs-primed seeds exhibited significantly elevated ROS levels compared to hydroprimed controls, with ROS predominantly localized in the embryo (Supplementary Figure S7A,B). This priming-triggered ROS burst likely primes pathways critical for the drought response and tolerance. To validate this, *N*-acetylcysteine (NAC) was introduced as a ROS scavenger to the CuO@SiO₂ NPs priming solution, and germination assay was conducted. NAC coapplication completely eliminated that CuO@SiO₂ NPs promoted seed germination; reductions in the germination speed, seedling vigor, seedling length, total dry weight, and ROS level were evident (Supplementary Figure S8). These findings confirm that the CuO@SiO₂ NPs-enhanced stress resilience is indeed ROS-dependent, directly linking embryonic ROS signaling to adaptive traits in later growth stages.

CuO@SiO₂ NPs Seed Priming Reprograms Seed Transcriptome. RNA-seq analysis was conducted to understand how CuO@SiO₂ NPs-generated ROS reprograms seed gene expression. Principal component analysis (PCA) reveals distinct transcriptomic profiles between CuO@SiO₂ NPs priming and hydropriming groups (Figure 4A), indicating that nanoscale treatment induced global transcriptional reprogramming in the seeds. A total of 6188 DEGs were identified, with 4045 upregulated and 2143 downregulated (Figure 4B). GO enrichment analysis reveals that these DEGs were predominantly associated with stress response pathways, including “response to external stimulus”, “response to gibberellin”, “response to hormone stimulus”, “response to biotic stimulus”, and “hormone-mediated signaling pathway” (Supplementary Figure S9). Several hormone-signaling-related genes, including cytokinin-*O*-glucosyltransferase (2.7-fold), auxin-responsive protein (4.9-fold), gibberellin 20 oxidase 4 (3.9-fold), gibberellin receptor (2.5-fold), and ethylene-responsive transcription factor (2.3-fold), were upregulated in CuO@SiO₂ NPs primed seeds (for the heatmap, see Figure 4C, and for more hormones-signaling-related genes, see Supplementary Table S2). Importantly, E3 ubiquitin–protein ligase, a central regulator of phytohormone signaling,³² was upregulated by 4.4-fold. These findings confirm the hypothesis that ROS-generation NPs could activate hormones and other signal transduction pathways. Physiological H₂O₂ concentrations in the extracellular space range have been reported to range from 0.1 to 1000 μ M.³³ It is likely that CuO@SiO₂ NPs in the extracellular space catalyze H₂O₂ transformation to •OH, and this •OH burst likely initiates redox-sensitive transcription factors, driving hormone-related transcriptional shifts.

RSA is tightly regulated by phytohormone signaling. To identify whether the activated hormone pathways trigger RSA-related gene activation, a comparative analysis was performed with a curated set of genes known to regulate RSA plasticity.³⁴ Significant overlap between NP-induced transcriptional changes and the established genetic regulators of RSA was identified. For example, the scarecrow (SCR) gene, known to be essential for the development of the maize primary root,³⁵ was upregulated (2.5-fold) in CuO@SiO₂ NPs-primed seeds (Figure 4C). In addition, several genes related to LR initiation and development were upregulated, including phospholipase A2 (PLA2, 1.6-fold), phospholipase C2 (PLAC2, 2-fold), polygalacturonase (PG, 1.7-fold), and expansin (EXP2, -4, -6, and -15, 1.6–4.2-fold) (Figure 4C). These cell-wall-modification genes facilitate cell wall loosening and separation for

outgrowth to occur from the lateral root primordium (LRP), which is the first step in the development of plant lateral roots.³⁶ Lateral root primordium 1 (LRP1) and indole-3-acetic acid, the most important transcriptional regulator of auxin signaling during LR development,³⁷ were also both upregulated (1.5- and 1.7-fold, respectively) upon priming, explaining the previously observed enhanced lateral roots in primed seedlings.

Apart from RSA-related genes, several drought-responsive genes were upregulated upon nanopriming, including dehydrins (DHNs) (2.2–3.8-fold), early light-inducible protein (ELIP) (4.6-fold), late embryogenesis abundant protein (LEA) (3.5-fold), desiccation-related protein (DRP) (3.2-fold), drought-induced protein 1 (Di1) (3-fold) (Figure 4C; for more drought-related genes, see [Supplementary Table S3](#)). Among them, DHNs stabilize biomolecules and membranes during dehydration stress.³⁸ ELIPs, located in thylakoid membranes, are known to protect photosynthetic machinery from photooxidative-induced damage.³⁹ LEA accumulates during seed desiccation and in response to the water deficit in vegetative plant tissues.⁴⁰ The activation of these drought-tolerant, and even desiccation-tolerant, genes significantly increases the drought tolerance of vegetative tissues.

Additionally, genes related to P uptake and transport were upregulated in seeds primed with CuO@SiO₂ NPs. Phosphate transporters belong to several different protein families, including the phosphate transporter (PHT) family and sulfate transporter-like (SULTR) family.⁴¹ The former are known to mediate inorganic phosphate (Pi) acquisition from soil or Pi translocation between tissues. Notably, the expressions of both PHT and SULTR were upregulated by 1.4- and 2.2-fold, respectively, in seeds primed with CuO@SiO₂ NPs (Figure 4C). This may contribute to the previously observed P uptake efficiency enhancement ([Supplementary Figure S5H](#)).

Given the observed enhancement of N uptake ([Supplementary Figure S5I](#)), the genes responsible for N uptake and assimilation were investigated, as well. Specifically, nitrate uptake is facilitated by the nitrate transporter (NRT) located in the plasma membrane.⁴² Subsequently, N assimilation initiates with two key enzymes: nitrate reductase (NR), which reduces nitrate to nitrite, and ferredoxin–nitrite reductase (NiR), which further reduces nitrite to ammonium.⁴³ Importantly, the expressions of NRT (2.4-fold), NR (3-fold), and NiR (2.2-fold) were all upregulated in CuO@SiO₂ NPs-primed seeds compared to hydroprimed seeds (Figure 4C), explaining the enhanced N uptake. Collectively, RNA-seq revealed the activation of genes related to RSA modulation, P/N/S uptake and assimilation, and drought tolerance in CuO@SiO₂ NPs-primed seeds, explaining the enhanced drought tolerance and P/N uptake in maize (Figure 4D).

Metabolic Reprogramming in Seeds. Metabolites are the critical products of cellular regulatory processes,⁴⁴ and the metabolomic profiles reflect dynamic biochemical changes in cells and tissues. The metabolite profile in maize seeds primed with CuO@SiO₂ NPs or water was analyzed. Using LC-MS/MS, 42 central metabolites were quantified. sPLS-DA revealed a clear separation between the two groups along principal component 1 (PC1; Figure 5A), indicating that CuO@SiO₂ NPs priming induced significant metabolic reprogramming in treated seeds. Notably, amino acids, sugars, organic acids, phenolic acids, and fatty acids were systematically elevated with CuO@SiO₂ NPs priming seeds compared to hydroprimed controls (Figure 5B,C). Amino acids increased by 7.6–105%,

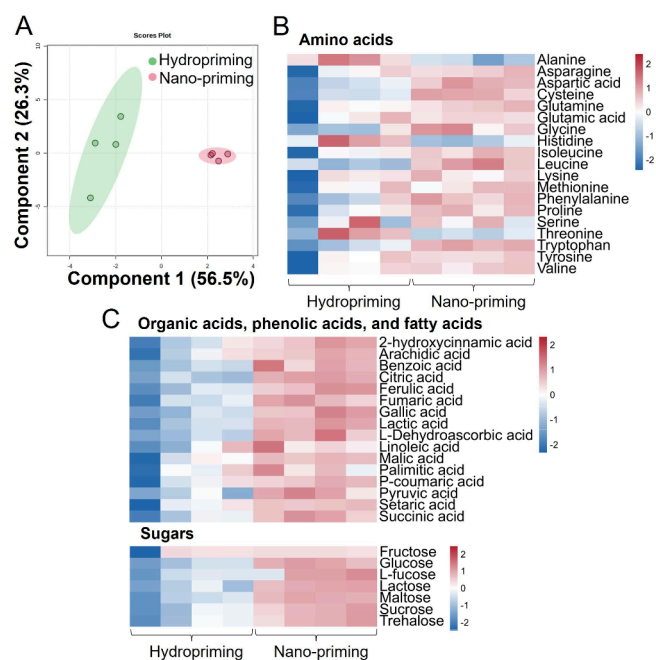


Figure 5. CuO@SiO₂ NPs seed priming reprogramming seed metabolites expression. (A) sPLS-DA plots showing cluster information between different treatment groups in maize seed primed by water or CuO@SiO₂ NPs for 24 h. (B and C) Heatmap showing the expression of all metabolites in seed.

while sugars exhibited a 20–24% increase. Organic acids exhibited the greatest changes, particularly citric acid (121%), pyruvic acid (89%), fumaric acid (53%), malic acid (49%), and succinic acid (49%) (Figure 5B,C). This systematic increase in seeds' primary metabolites suggests that CuO@SiO₂ NPs priming accelerated the catabolism of seed stored reserves, a process fueled by respiratory metabolism. We further measured ATP, the end product of respiration, and found that its level was significantly increased by 24.1% ($p < 0.05$) in CuO@SiO₂ NPs-primed seeds compared to the control. This indicates an enhanced respiratory energy metabolism, which explains the observed acceleration in seed germination ([Supplementary Figure S10](#)). Additionally, ROS-scavenging metabolites were also significantly enriched in CuO@SiO₂ NPs primed seeds, including 2-hydroxycinnamic acid (111%), ferulic acid (200%), gallic acid (47%), L-dehydroascorbic acid (50%), and *p*-coumaric acid (166%) (Figure 5C). The upregulation of these metabolites likely protects cells from imbibition-induced membrane damage, further supporting the priming efficacy of CuO@SiO₂ NPs.

Environmental Implications. The current work demonstrates that a simple seed priming strategy employing engineered ROS-generating CuO@SiO₂ NPs can simultaneously enhance the resilience of maize to both water and P deficiency by activating genes associated with drought tolerance, RSA, and P/N/S uptake and assimilation. Importantly, this approach significantly increased the maize yield under both optimal and drought-stressed conditions. By simultaneously boosting crop yield while reducing reliance on water and P fertilizer, this innovative nanoenabled strategy provides dual benefit: enhancing climate-resilient crop production and promoting sustainable agriculture through efficient resource use.

CuO@SiO₂ NPs hold great promise for real-world applications due to their low-cost, ecofriendly profile, and multifunctional nature. First, the estimated application cost is \$0.62–1.15 per acre, calculated based on material cost (\$0.5/g), dosage (20 mg/L), use amount (100 g of seeds in 0.5 L of CuO@SiO₂ NPs suspension), and planting density (49850–92100 plants/acre), demonstrating the economic viability of this approach for farmers, particularly smallholders. Second, the core–shell structure ensures a high Cu utilization efficiency by exposing only a small amount of active Cu on the surface, thereby reducing potential environmental risks. In addition, the seed priming application minimizes the potential for environmental release that can occur with the broadcast spraying of conventional agrochemicals. Third, beyond their primary design as ROS-generating nanostimulants, CuO@SiO₂ NPs have additional functions. CuO NPs have been reported to have broad-spectrum antibacterial activities,⁴⁵ sometimes surpassing that of silver NPs,⁴⁶ suggesting a role as a nanopesticide. Concurrently, the sustained release of Cu ions acts as a nanofertilizer, supplying a critical micronutrient for enzyme function. After the CuO shell dissolves, the SiO₂ core can release Si, a beneficial element for strengthening cell walls.^{47–49} Together, this multifunctionality positions CuO@SiO₂ NPs as a triple-action platform that combines the benefits of a nanostimulant, nanopesticide, and nanofertilizer into a single application.

We primarily attribute the improved drought tolerance, P acquisition, and maize yield to direct stimulation by CuO@SiO₂ NPs, but we cannot exclude potential contributions from plant-associated microbes. We previously observed that CuO@SiO₂ NPs seed priming accelerates respiration and catabolism of seed reserves, which increases the abundance of seed metabolites, including sugars, amino acids, organic acids, and phenolics. This may result in more metabolites being released into the spermosphere, the zone surrounding seeds where interactions among the germinating seed, microbial community, and soil occur.⁵⁰ This could fuel microbial growth, and such early recruitment may then propagate to the rhizosphere, conferring benefits, such as improved plant growth, drought tolerance, and P acquisition. These hypotheses and the real-world efficacy of this approach will be investigated in future multilocation field trials.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.5c11193>.

Details of the supporting methods (Supplementary Texts S1 and S2), historical weather data and gene-expression-level data (Supplementary Tables S1–S3), pre-experimentation of the priming concentration (Supplementary Figure S1), seed priming performance, P and nitrate acquisition, Cu content in maize seeds, and ROS level (Supplementary Figures S2–S8), and GO enrichment and ATP content (Supplementary Figures S9 and S10) (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

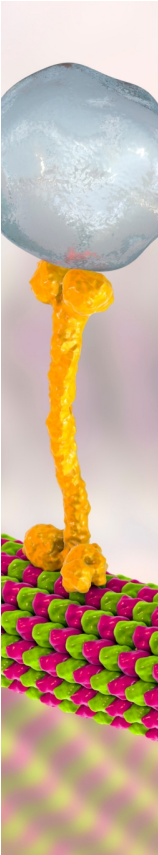
This work is supported by the Independent Innovation Fund for Agricultural Science and Technology in Jiangsu Province [CX (23)3015].

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