

## Supplementary information

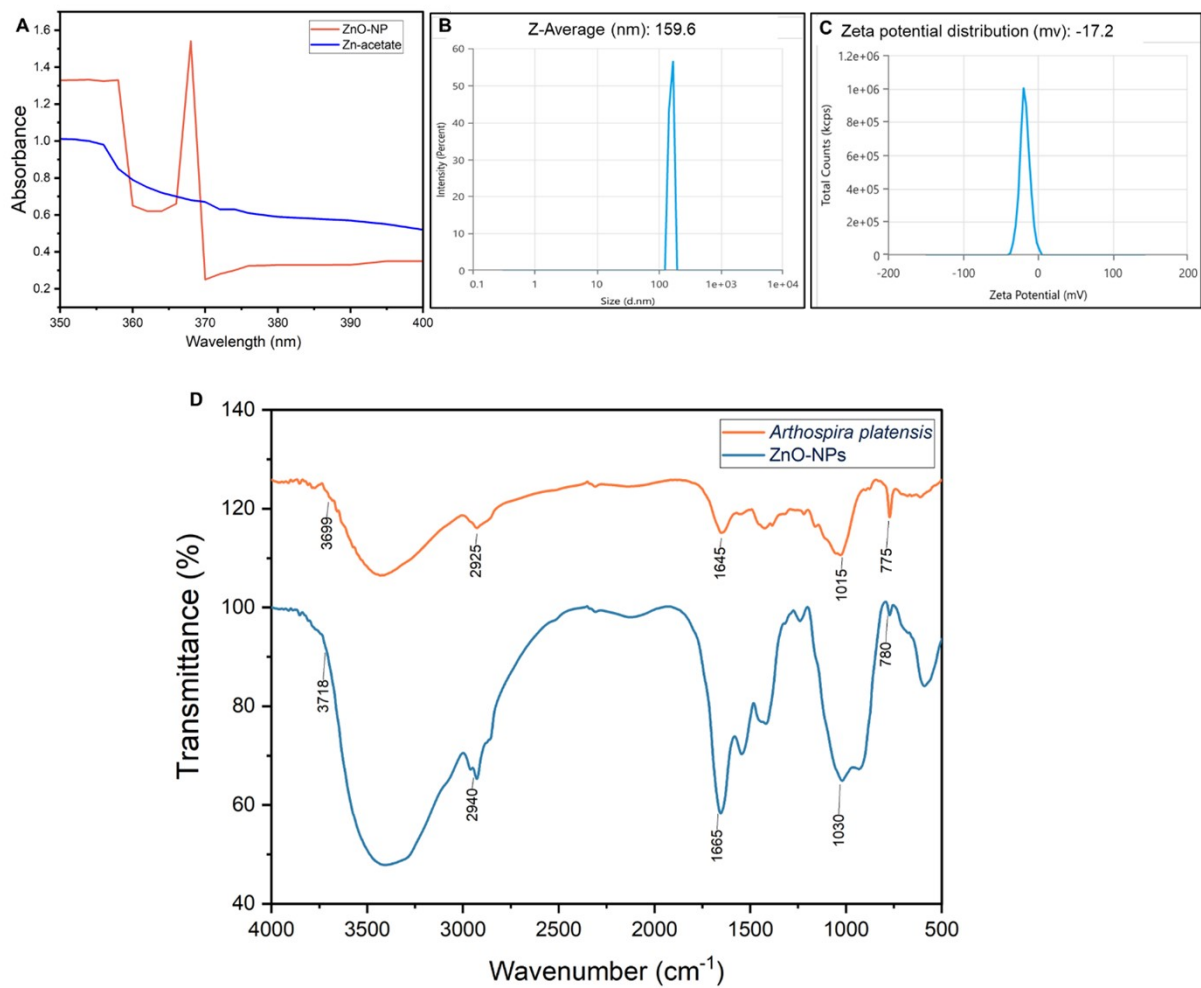
### **Phycofabricated zinc oxide nanoparticles and hydrogen sulfide confer drought tolerance in rice (*Oryza sativa* L.) by modulating abscisic acid signaling and redox homeostasis**

**Abir Das,<sup>a</sup> Sayan Pal,<sup>a</sup> Dip Pal,<sup>b</sup> Narottam Dey,<sup>b</sup> Malay Kumar Adak<sup>\*a</sup>**

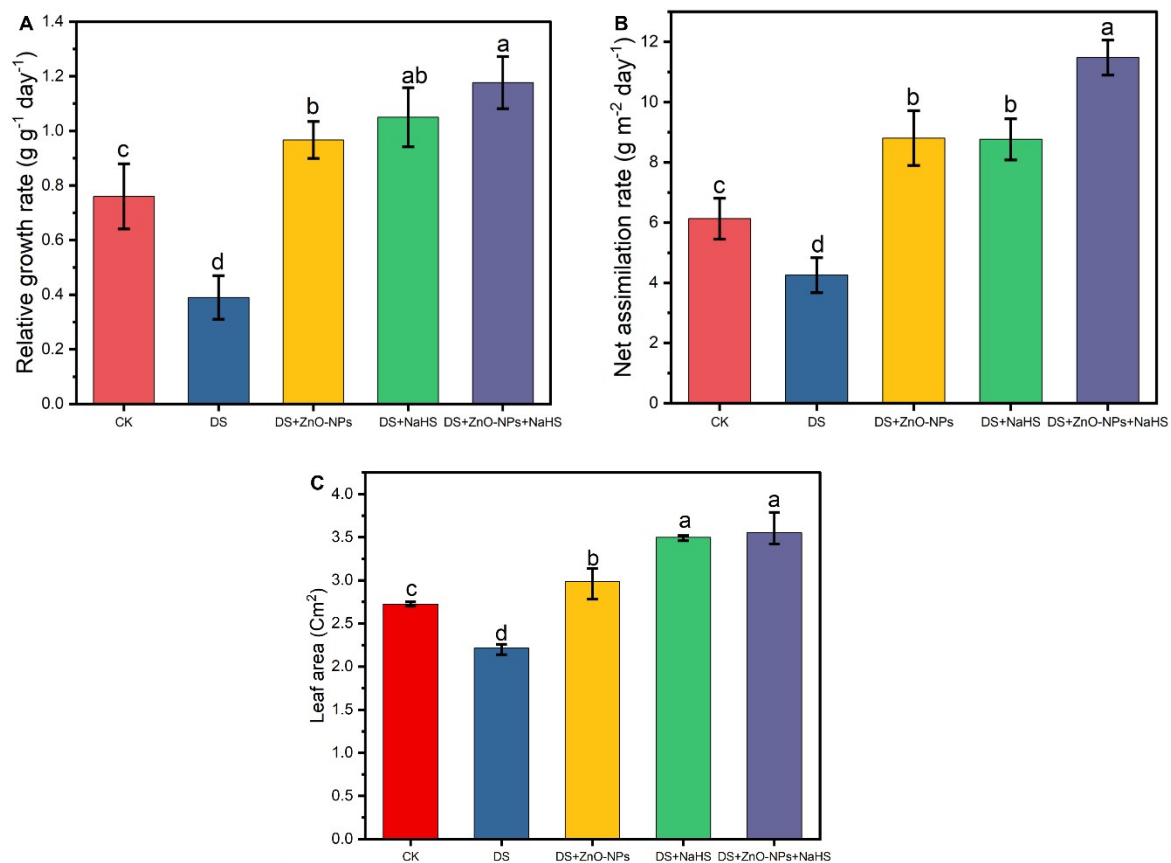
*<sup>a</sup>Plant Physiology and Molecular Biology Research Unit, Department of Botany, University of Kalyani, Kalyani, West Bengal 741235, India*

*<sup>b</sup>Rice Biotechnology Laboratory, Department of Biotechnology, Visva-Bharati, Santiniketan, West Bengal, 731235, India*

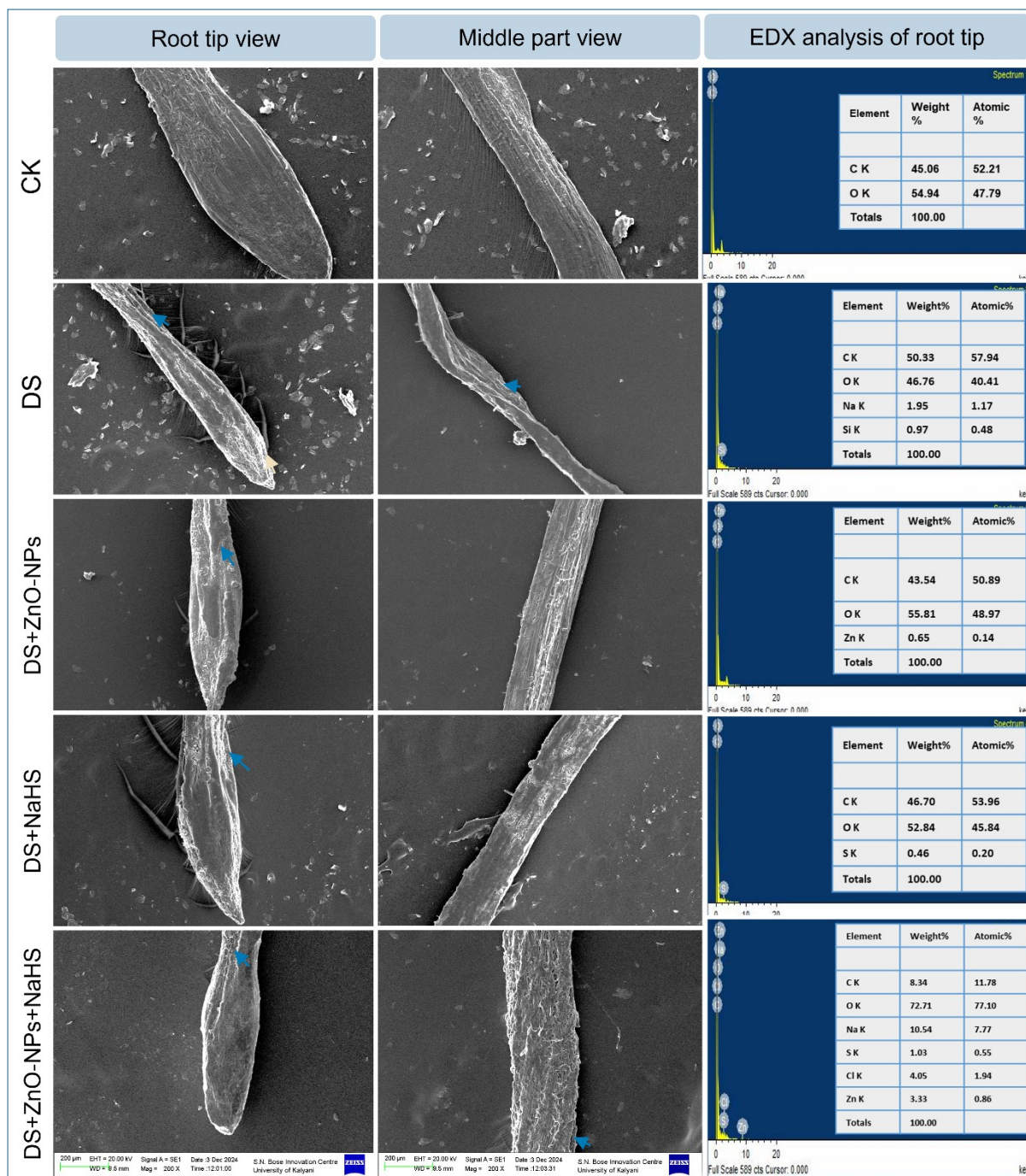
\*Corresponding Email: [malay009@klyuniv.ac.in](mailto:malay009@klyuniv.ac.in) (M.K.A)



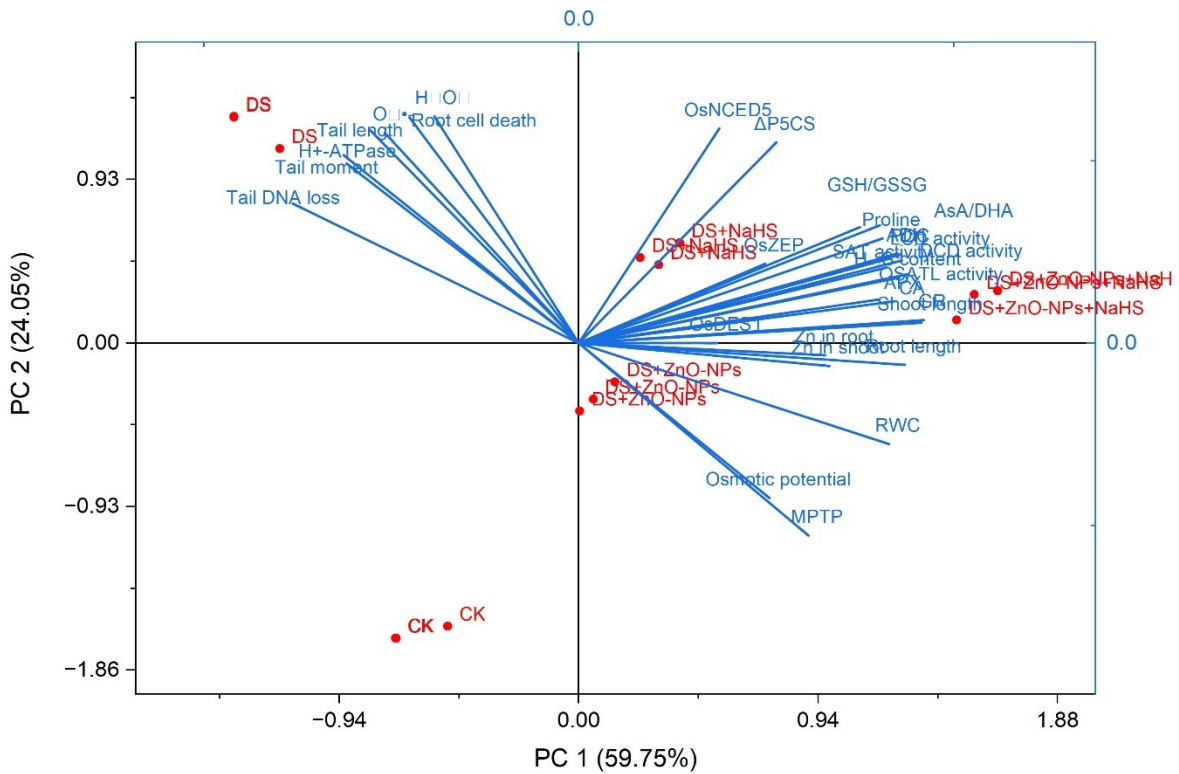
**Fig. S1** Phycofabricated ZnO-NPs characterization through opto-physical techniques: (A) UV-vis spectroscopy, (B) DLS analysis, (C) Zeta ( $\zeta$ ) potential, and (D) FTIR spectroscopy.



**Fig. S2** Changes in growth attributes of rice seedlings. (A) relative growth rate, (B) net assimilation rate, and (C) leaf area in cv. Swarna Sub1 showing variations for each stress treatment under PEG-induced DS for 14-days. Data represented as mean  $\pm$  SE (n = 3) with statistical significance was analyzed by One way ANOVA; small lettering in each bars indicate Tukey test.



**Fig. S3** Plant responses to DS were initially observed through changes in root activity. Surface morphology and mineral distribution, analyzed through SEM imaging and EDX mapping, showed distinct variations under each stress treatment during 14-days of PEG-induced DS.



**Fig. S4** Two-dimensional scatter diagram with principal component analysis depending on the first and second principal components of different physiological attributes considered in the experiment.

### Supplementary methods and materials

To optimization of hydrogen sulfide (H<sub>2</sub>S) concentration under drought, various NaHS concentrations of were tested based on percent germination, seedling vigor index (SVI), seedling fresh and dry weights, and seedling water content (SWC). Root and shoot lengths (cm), as well as fresh and dry weights (g), were measured from five seedlings per replicate. Dry weights were obtained after oven-drying samples at 80 °C for 72 hours, according to Black & Pritchard (2002). Here, FW is fresh weight and DW is dry weight of seedlings.

SVI was computed as:

$$SVI = (\text{seedling length} \times \text{percentage of germination}) / 100$$

SWC was calculated using the formula:

$$SWC = (FW - DW) / FW \times 100$$

**Table S1** Effect of different concentration of H<sub>2</sub>S priming (6 hours) on rice plant growth parameters

Concentration	GP	SL	SVI	SWC
Control	49.15±3.12d	17.7±3.15d	11.35±1.21d	23.91±1.51c
H <sub>2</sub> S 10 µM	51.25±6.38d	31.1±2.51b	27.17±2.35b	31.3±2.17b
H <sub>2</sub> S 100 µM	93.25±5.48a	44.1±3.37a	36.25±2.27a	39.88±3.34a
H <sub>2</sub> S 200 µM	72.5±4.60b	25.2±1.08c	19.15±0.99c	26.11±1.21c
H <sub>2</sub> S 300 µM	60±5.75c	27.6±2.31c	20.58±3.24c	18.67±1.33d

All mean values are presented as ± SE (standard error) based on three replicates. Means not sharing a common letter differ significantly at  $p \leq 0.05$ . Here, GP: germination percentage, SL: seedling length, SVI: seedling vigor index, SWC: seedling water content

**Table S2** Oligonucleotide primer sequences used for RT-qPCR analysis

Gene name	Primer sequences (5' → 3')	
	Forward sequence	Reverse sequence
<i>Ubiquitin5 (UbiQ5)</i>	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
<i>OsZEP</i>	TCTAGGAGGAAACAGCACGA	CCAATGCATCGTCATCCTC
<i>OsNCED5</i>	ACATCCGAGCTCCTCGTCGTGAA	TTGGAAGGTGTTTTGGAATGAACCA
<i>OsDES1</i>	TCGAGTCAGTCAGATATGAAGCT	TGTAACCTTGGTACCAACATCTCT

## Reference

Black, M.; Pritchard, H. W., Eds. Desiccation and Survival in Plants: Drying without Dying, 1st ed.; CABI Publishing: UK, 2002. <https://doi.org/10.1079/9780851995342.0000>.