Electronic Supplementary Information

Dual Nanozyme Characteristics of Iron Oxide Nanoparticles Alleviate Salinity Stress and Promote Growth of an Agroforestry Tree, *Eucalyptus tereticornis* Sm.

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S1. Materials Used

Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), 6-benzylaminopurine (BAP), 1-naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA) were procured from Sigma-Aldrich (USA). Ammonia solution ($NH_3.H_2O$), hydrogen peroxide (30%w/v H_2O_2) and o-Dianisidine were procured from Loba-Chemie Pvt. Ltd, India. Following chemicals were procured form were procured from Hi Media Laboratories Pvt. Ltd. (Mumbai, India); Ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), calcium chloride dehydrate ($CaCl_2.2H_2O$), potassium dihydrogen phosphate (KH_2PO_4), manganese sulphate monohydrate ($MnSO_4.H_2O$), zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), boric acid (H_3BO_3), potassium iodide (KI), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$), copper sulphate pentahydrate ($CuSO_4.5H_2O$), cobaltous chloride hexahydrate ($CoCl_2.6H_2O$), ethylenediaminetetraacetic acid ferric sodium salt (Fe-EDTA), thiamine hydrochloride, nicotinic acid, pyridoxine hydrochloride, glycine, myo-inositol, sucrose, agar, hydrochloric acid (HCl),sulphuric acid (H_2SO_4), phenol, potassium hydroxide (KOH), sodium hydroxide (NaOH), sodium chloride (NaCl), sulphosalicylic acid, ninhydrin; 3,5-dinitrosallicylic acid (DNS), potassium sodium tartrate, sodium carbonate (Na_2CO_3), L-cysteine hydrochloride, Folin-ciocalteu's phenol reagent, polyvinyl pyrrolidone (PVP), trichloroacetic acid (TCA), thiobarbituric acid, potassium hydrogen phosphate (K_2HPO_4), L-proline, bovine serum albumin (BSA), D-glucose, nitro blue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), EDTA-disodium, riboflavin, L-ascorbic acid, methionine, nitro blue tetrazolium (NBT), acetone, methanol, glacial acetic acid and toluene. Ethanol was obtained from Bengal Chemicals and Pharmaceuticals Ltd. (Kolkata, India).All reagents were of analytical grade and used without any additional purification. All aqueous preparations were carried out in deionized water.

S2. Characterization Details

The absorption spectrums were measured by the UV-Vis spectrophotometer (UV-2600, Shimadzu Corp., Japan). A Cary 630 spectrometer (Agilent Technologies, USA) over the range of 400–4000 cm^{-1} was used to obtain Fourier transform infrared (FT-IR) spectra of as synthesized IONPs. Evaluation of magnetic properties was done using a magnetometer (7407 Lake Shore Cryotronics Inc., USA) at room temperature with an applied field between −10,000 and 10,000 Oe. The sample crystallinity of IONPs was evaluated using X-ray diffractometer (PANalytical X-pert Pro, The Netherlands) with CuKα radiation (λ = 1.5406 Å) over a scanning range (2θ) from 20 to 70.The structural morphology of IONPs was studied using high resolution transmission electron microscopy (HR-TEM, JEOL JEM-2100, Japan). The hydrodynamic size and zeta potential of IONPs was determined by Zetasizer (Malvern ZEN-3600, Malvern Panalytical).

Table S1: The hydrodynamic size and zeta potential of IONPs, as synthesized and after introducing in the MS medium with varied concentration. The values are represented as Mean±SE of three independent replicates.

Sample	*Hydrodynamic size*	*Zeta potential*
IONPs (in deionized water)	74±4.2 nm	-23.7±1.2 mV
10 ppm (MS medium)	81±5.7 nm	-22.2±0.8 mV
25 ppm (MS medium)	85±6.3 nm	-20.8±1.1 mV
50 ppm (MS medium)	94±8.5 nm	-18.9±0.5 mV

Table S2: The effect of various concentration (0-50 ppm) of Fe-EDTA on shoot length (fold increase), shoot biomass (fold increase), number of roots per shoot and root length (fold increase) of *E. tereticornis* growing under normal and stress conditions. Data were represented as mean±SE of three independent replicates. Mean values followed by different letters (Lower case: normal; Uppercase: stress conditions) are significantly different at $P<0.05$ by LSD

Condition	**Fe-EDTA (ppm)**					
	10	**20**	**25**	**30**	**40**	**50**
Shoot length (fold increase)						
Normal	$0.99^{a}\pm0.003$	$0.96^{ab}\pm0.017$	$0.92^{abc}\pm0.018$	$0.89^{bc}\pm0.009$	$0.86^{c}\pm0.003$	$0.77^{d}\pm0.041$
Stress	$0.94^{A}\pm0.027$	$0.89^{A}\pm0.015$	$0.83^{B}\pm0.013$	$0.78^{B}\pm0.021$	$0.72^{C}\pm0.004$	$0.69^{C}\pm0.006$
Shoot biomass (fold increase)						
Normal	$0.93^{a}\pm0.005$	$0.88^{ab}\pm0.015$	$0.83^{b}\pm0.019$	$0.70^{c}\pm0.018$	$0.63^{c}\pm0.021$	$0.45^{d}\pm0.045$
Stress	$0.74^{A}\pm0.028$	$0.68^{A}\pm0.034$	$0.51^{B}\pm0.039$	$0.44^{BC}\pm0.052$	$0.35^{CD}\pm0.012$	$0.29^{D}\pm0.019$
No. of roots per shoot (fold increase)						
Normal	$0.96^{a}\pm0.009$	$0.62^{b}\pm0.025$	$0.34^{c}\pm0.040$	0^{d}	0^{d}	0^{d}
Stress	$0.77^{A}\pm0.043$	$0.55^{B}\pm0.024$	0^{C}	0^{C}	0^{C}	0^{C}
Root length (fold increase)						
Normal	$0.92^{a}\pm0.007$	$0.73^{b}\pm0.009$	$0.43^{c}\pm0.036$	0^{d}	0^{d}	0^{d}
Stress	$0.67^{A}\pm0.036$	$0.46^{B}\pm0.092$	0^{C}	0^{C}	0^{C}	0^{C}

Table S3: Sequences of the primers used for qRT-PCR studies

Gene	Sequence (5’------3’)	
HKT1	Forward	TTGCCTGAACTCGCAGGTAG
	Reverse	ACTGCGGCAACTCCTAGAAC
NHX1	Forward	GCCGCATCAGTTGTTCTGTT
	Reverse	CCAAAACTCCGAGCATCGTG
SOS1	Forward	ACACCACACGTAGCTCAAG
	Reverse	TGCCGAAAATGCTGAGTTGC
SOD	Forward	GGCTTGCATACAAACCTGAA
	Reverse	CTGACTGCTTCCCATGACAC
CAT	Forward	GTCGATTGGTGTTGAACAGG
	Reverse	AGGACGACAAGGATCAAACC
POX	Forward	TTAGGGAGCAGTTTCCCACT
	Reverse	AGGGTGAAAGGGACATCAG
β-actin	Forward	AGGAGCATCCTGTCCTCCTAA
	Reverse	CACCATCACCAGAGTCCAACA
(EF)1-α	Forward	GATGGTCAGACCCGTGAACA
	Reverse	CCTTGGAGTACTTCGGGGTG
Sec3A	Forward	GCTTGCACACGCCATATCAAT
	Reverse	TGGATTTTACCACCTTCCGCA

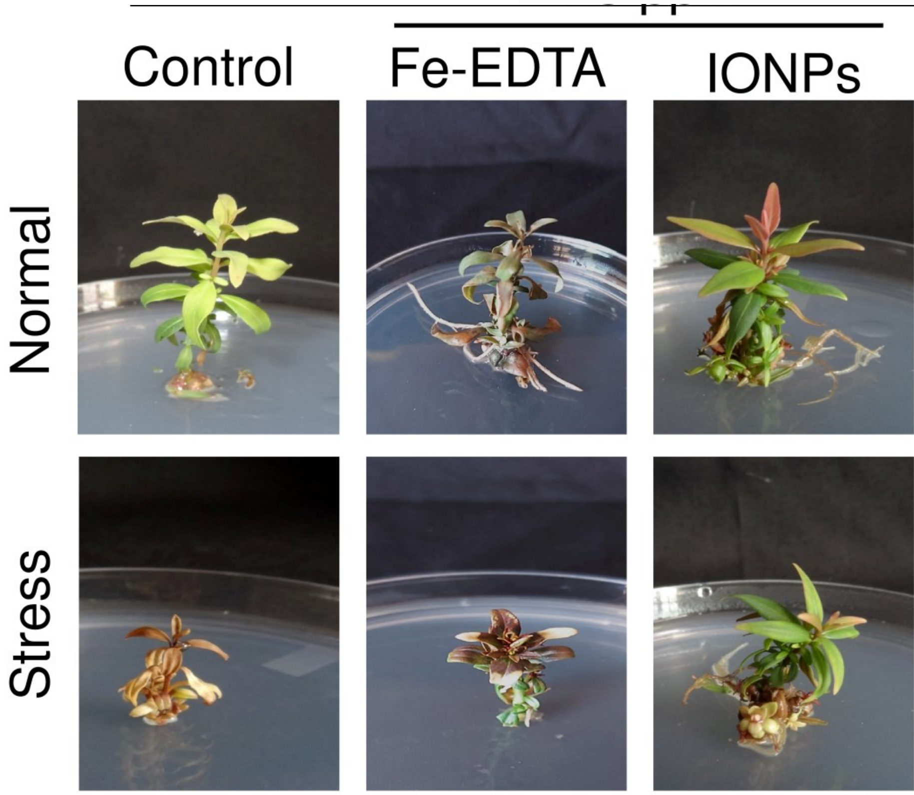

Figure S1: Effect of 25 ppm Fe-EDTA and IONPs on the shoot cultures of *Eucalyptus tereticornis* growing under normal and salt stress conditions.

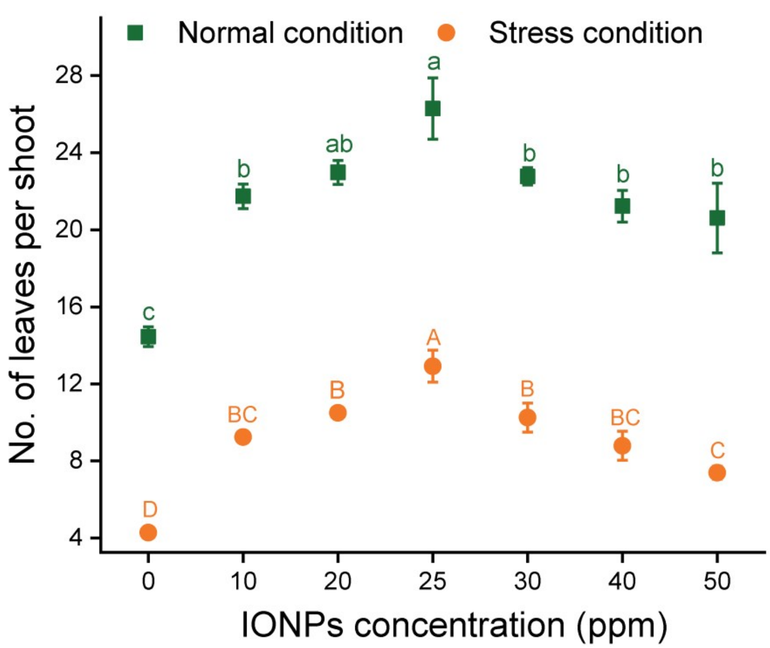

Figure S2: Effect of various concentrations (0-50 ppm) of iron oxide nanoparticles (IONPs) on the number of leaves per shoot of *Eucalyptus tereticornis* growing under normal and stress conditions. Data were recorded after 30 days of culture and the error bars represent the standard error of three different experiments. Mean values followed by different letters (Lowercase:Normal; Uppercase: Stress) are significantly different at $P<0.05$ by LSD.

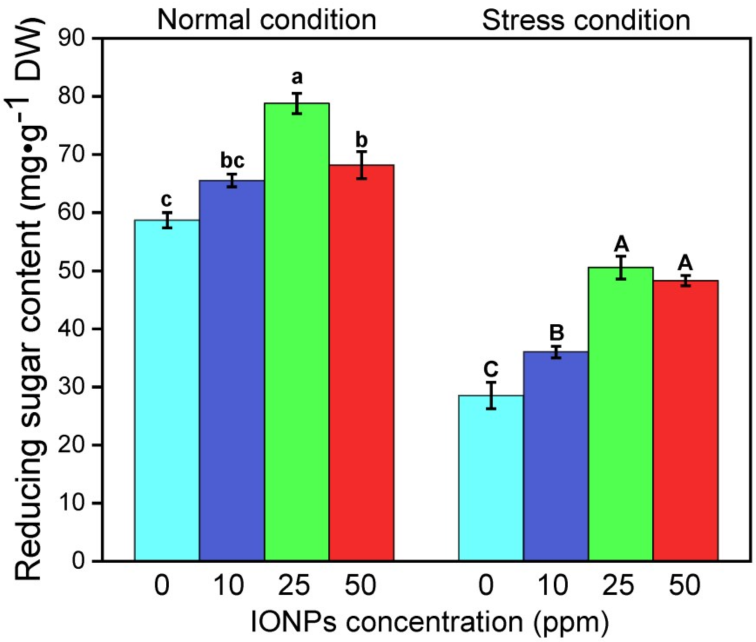

Figure S3: Effect of various concentrations (0, 10, 25, and 50 ppm) of iron oxide nanoparticles (IONPs) on the reducing sugar content in the cultures of *E. tereticornis* growing under normal and stress conditions. Data were recorded after 30 days of culture and error bar represent standard error of three different replicates. Mean values followed by different letters (Lowercase: Normal; Uppercase: Stress) are significantly different at $P<0.05$ by LSD.