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Research Article

ASSESSMENT OF ANTIOXIDANT ENZYME RESPONSES IN HOST (SESAMUM INDICUM L.; PEDALIACEAE) FOLLOWING NANOPARTICLES (COPPER, COPPER OXIDE AND COPPER DOPED ZINC OXIDE) EXPOSURE

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ABSTRACT

Synthesized and characterized nanoparticles-NPs (copper, copper oxide and copper doped zinc oxide) are exposed to host (*Sesamum indicum* L.; family: Pedaliaceae) for assessment of plant defense responses against oxidative stress with an objective to provide insight on stress minimizing reaction kinetics. The study also encompasses NPs accumulation in seedling ash in the form of cations using Atomic Absorption Spectroscopy (AAS). Results highlight the following: 1) Cu- and CuO-NPs exhibit non-coordinated antioxidant enzyme responsiveness by the host suggesting prevalence of cellular stress environment;while, Cu-doped ZnO-NPs show ground level reactivity and 2) antioxidant enzyme responses corroborates with NPs accumulation.

INTRODUCTION

Nanomaterials engineered structures residing in the nanoscale region [1], possesses characteristics like high surface to volume ratio [1], enhanced penetration ability, potent surface reactivity [2] among others, are significantfor nano-bio interaction [3] and toxicological impact on host system [4]. Plantsspecies, primary site of bio-reactivity with soil deposited nanoparticles-NPs (soil act as sink) that are anthropogenically released in the environmental inter-collegium, can be explored (due to applicational simplicity and cost effectivity) as model for assessment of NPs mediated stress and toxicity. However, stress accumulation induced by NPs in host system can activate their own defense mechanism by triggering anti-oxidant enzymes [5]. Therefore, assessment of antioxidant enzyme responsiveness can provide insight on stress minimizing reaction kinetics by the host. With the view to it, present investigation highlights the antioxidant enzymes (ascorbate peroxidase-APX, total superoxide dismutase-tSOD, monodehydroascorbate reductase-MDAR, glutathione reductase-GR and glutathione-S-transferase-GST) responses in host (*Sesamum indicum* L.; Family: Pedaliaceae; oil-seeds of commerce) following the applications of NPs (copper-Cu, copperoxide-CuO and Cu-doped zinc oxide-ZnO) on a comparative basis.

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The study also encompasses quantification of NPs accumulation (assessed by Atomic Absorption Spectroscopy-AAS) in host with an objective to evaluate if there exists any inter-relationship with the defense enzymes kinetics.

MATERIALS AND METHODS

Germplasm

Breeder seeds of *Sesamum indicum* L. var. B67 (moisture content: 7.40%) was collected from Pulse and Oil Seed Research Station, Govt. of West Bengal, Berhampore, India.

NPs synthesis and characterization

Copper (Cu), copper oxide (CuO) and copper doped zinc oxide (Cu-doped ZnO) nanoparticles (NPs) were prepared following wet chemical co-precipitation techniques [6] and were subsequently characterized using different opto-physical instrumentation (UV-vis, FTIR, XRD, DLS-zeta potential and FESEM) techniques. Results consisting particle size and morphology (size: Cu-NPs - metallic core- 18.37nm±6.39, gelatinous shell-64.23nm±22.37; CuO-NPs - 56.47nm±4.20; Cu-doped ZnO-NPs - 79.2nm±27.48; shape: Cu-NPs - face centered cuboid geometry; CuO-NPs - spherical to ellipsoidal geometry and Cu-doped ZnO-NPs - wurtzite hexagonal nanorod) are reported by Das et al. [6]. Bulk materials (without employment of capping agents) were also prepared for comparison.

Treatments

About two hundred dry seeds were given in each dose (1.0, 2.0 and 4.0 µg ml^{-1}; 3 and 6 h durations) of NPs treatments. Bulk materials werealso prepared (1.0 µg ml^{-1}, 3h) and kept for assessment as control along with dry control for comparison.

Assessment of NPs uptake in seedlings

About 0.2 g seedlings from each treatment (including bulk and dry control) were taken as primary dry mass for Atomic Absorption Spectroscopic (AAS) analysis. Among the six replicas of each treatment, three were maintained as seed-coat containing replica (SCR); while, in rest three seed-coat remnants were removed, surface cleaned with ddH_2O and considered as seed-coat devoid replica (SDR). Seedling ash was produced following heating the initial dry mass under muffle furnace (400˚C). Ashes were then subjected to acid digestion (sulphuric acid: nitric acid: perchloric acid::3:3:1) followed by volume reduction to 5 ml under fume hood. Remaining acid digested samples were then diluted (volume made upto 25 ml by deionized water) and analyzed using AAS (Agilent technologies) with Cu^{2+} and Zn^{2+} detection wavelength at 520 nm and 213.9 nm respectively.

Measurement of antioxidant enzyme reactivity

Ascorbate peroxidase (APX) analysis

Enzyme (EC 1·11·1·11) was extracted from 0.4 g ten days old frozen seedlings following homogenization in 50 mM potassium phosphate buffer (pH 7.8, consisting 4 mM ascorbate, 2M ethylenediaminetetraacetic acid- EDTA, 2 mM dithithrietol- DTT and 2% w/v polyvinylpyrroidone- PVP).

APX kinetics was measured following Nakano and Asada [7]. Pre-enzyme reaction mixtures were prepared following addition of 800 µL phosphate buffer (50 mM, pH 7.0), 200 µL EDTA (1 mM), 40 µL H_2O_2 and 760 µL ascorbate (1 mM). Enzymatic reaction was initiated by adding 100 µL enzyme extract into pre-reaction solution. Rate of absorption efficiency at 290 nm (Ɛ=2.8 mM^{-1} cm^{-1}). APX reactivity was represented as µmol ascorbate oxidation in unit time and protein concentration.

Measurement of total superoxide dismutase (tSOD), monodehydroascorbate reductase (MDAR) and glutathione reductase (GR) reactivity

Antioxidant enzyme isolation was performed from ten days old seedlings (0.4 g). For the purpose, seedlings were homogenized in 50 mM phosphate buffer (pH 7.8, containing 2mM EDTA, 2mM DTT and 2% PVP). Resultant suspensions were centrifuged at 12,000 rpm for 30 min at 40˚C. Upper aqueous phase was taken as enzyme source.

tSOD activity

Total SOD (EC 1·15·1·1) activity was analyzed following Beyer and Fridovich [8]. Pre-reaction solution was prepared by adding 880 µL phosphate buffer (50 mM, pH 7.8), 50 µL L-methionine (10 mM), 30 µL triton X-100 (0.05% v/v), 30 µL nitroblue tetrazolium (NBT, 60 µM) and 20 µL enzyme. Reaction commences following addition of riboflavin and light exposure (20W lamp) for 7 min. Absorbance was recorded at 560 nm and enzyme reactivity was represented as unit per mg of protein (U mg^{-1}).

MDAR reactivity

MDAR (EC 1·6·5·4) kinetics was assessed as per Miyake and Asada [9] and was expressed as µmol NADPH oxidized per unit time (min) and protein concentration.

GR kinetics

GR (EC 1·8·5·1) assay was performed as per Carlberg and Mannervik [10]. For the purpose, pre-reaction solution (200 mM phosphate buffer, 2 mM EDTA, 2 mM NADPH and 20 mM oxidized glutathione, pH 7.0) was mixed with enzyme extract. Sample absorbance was recorded at 340 nm. Rate kinetics was measured following extinction co-efficient of NADPH (6.2 mM^{-1} cm^{-1}).

Extraction and estimation of glutathione-S-transferase

For enzyme (EC 2·5·1·18) extraction, ten days old seedlings were ground in extraction buffer (100 mM phosphate buffer, 2 mM EDTA and 0.2% PVP, pH 7.0). Homogenized suspensions were centrifuged at 12,000 rpm for 30 min (4˚C). Clear supernatants were taken as enzyme source.

GST reactivity was determined as per Aebi [11]. For the purpose, pre-reaction mixtures was prepared using potassium phosphate buffer (50 mM,pH 7.5) and 1 mM 1-chloro-2,4 dinitobenzene (cDNB).

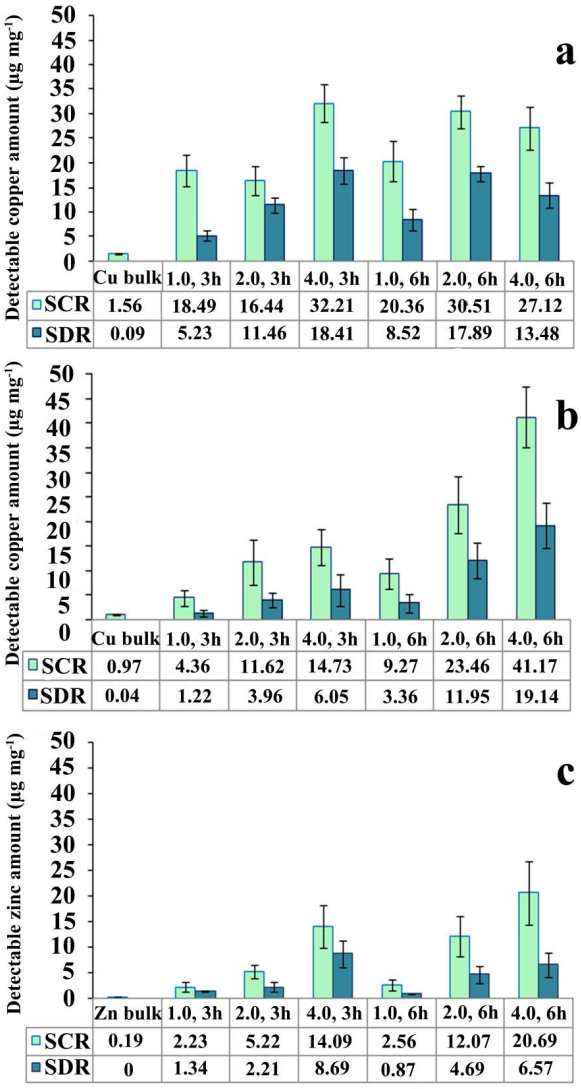

Figure 1 (a–c) NPs accumulation in seedling ash of *Sesamum indicum*.

Reaction was initiated by adding 1 mM reduced glutathione (GSH) and 100 µL enzyme extract. Increase in absorption efficiency was reduced at 340 nm.

Statistical analysis

Duncun's t-test is performed between controls and treatments using IBM SPSS software v20.

RESULTS AND DISCUSSION

Assessment of NPs accumulation

In relation to dry and bulk controls, NPs accumulation (in the form of Cu^{2+} and Zn^{2+} metallic cations) in seedling ash exhibits significant (p<0.05) enhancement in both seed-coat containing replicas (SCR) and seed-coat devoid counterparts (SDR); however, the uptake is higher in SCR than SDR (Fig 1a–c) therebyindicating that hard seed coat actsas potent inhibitor against NPs internalization. Seed-coat acting as barrier for NPs uptake is reported earlier [12,13]. In the present investigation, NPs uptake is mostly found to bedose dependent (excepting: 2.0µg ml^{-1}, 3h and 4.0 µg ml^{-1}, 6h Cu-NPs). Reduction in Cu^{2+} uptake in 4.0 µg ml^{-1}, 6h of Cu-NPs can be attributed to particle agglomerization as the possible consequence of gelatinous covering of the synthesized NPs [6].

Uptake in seedling ash is found to bein the order of CuO-NPs> Cu-NPs>ZnO-NPs (Fig 1a–c).

Particle morphology, aggregation tendency, monodispersion ability, surface charge potentiality, among others are reported to be the determining factors for NPs accumulation in the biological system [14-16].

Measurement of antioxidant responsiveness

NPs internalization in seedlings results in varyingdegree of oxidative stress induction[17]. Das et al. [6] quantified the amount of oxidative stress in the form of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) by the presently studied NPs in *S. indicum*, and it is found in the order of CuO-NPs>Cu-NPs>ZnO-NPs. Such oxidative stress may be due to nano-dimension of the synthesized NPs.

In the present investigation, NPs inducedefensein the form of antioxidant enzymes effectivityin host in a differential manner. The enzyme kinetics is studied in relation to dry and bulk controls. The studied enzymes demonstrate base level reaction kinetics in alltreatments of Cu-doped ZnO-NPs (excepting tSOD- 4.0 µgml^{-1}, 3h) suggesting insignificant effect on host defense response.

APX activity enhances significantly (p<0.05) in Cu- and CuO-NPs treatments mostly (excepting: 2.0 µg ml^{-1} and 4.0 µg ml^{-1}, 6h Cu-NPs) in a dose dependent manner (Fig 2 a–c). At comparative doses, enzyme activity is more pronounced in CuO-NPs than Cu-NPs.

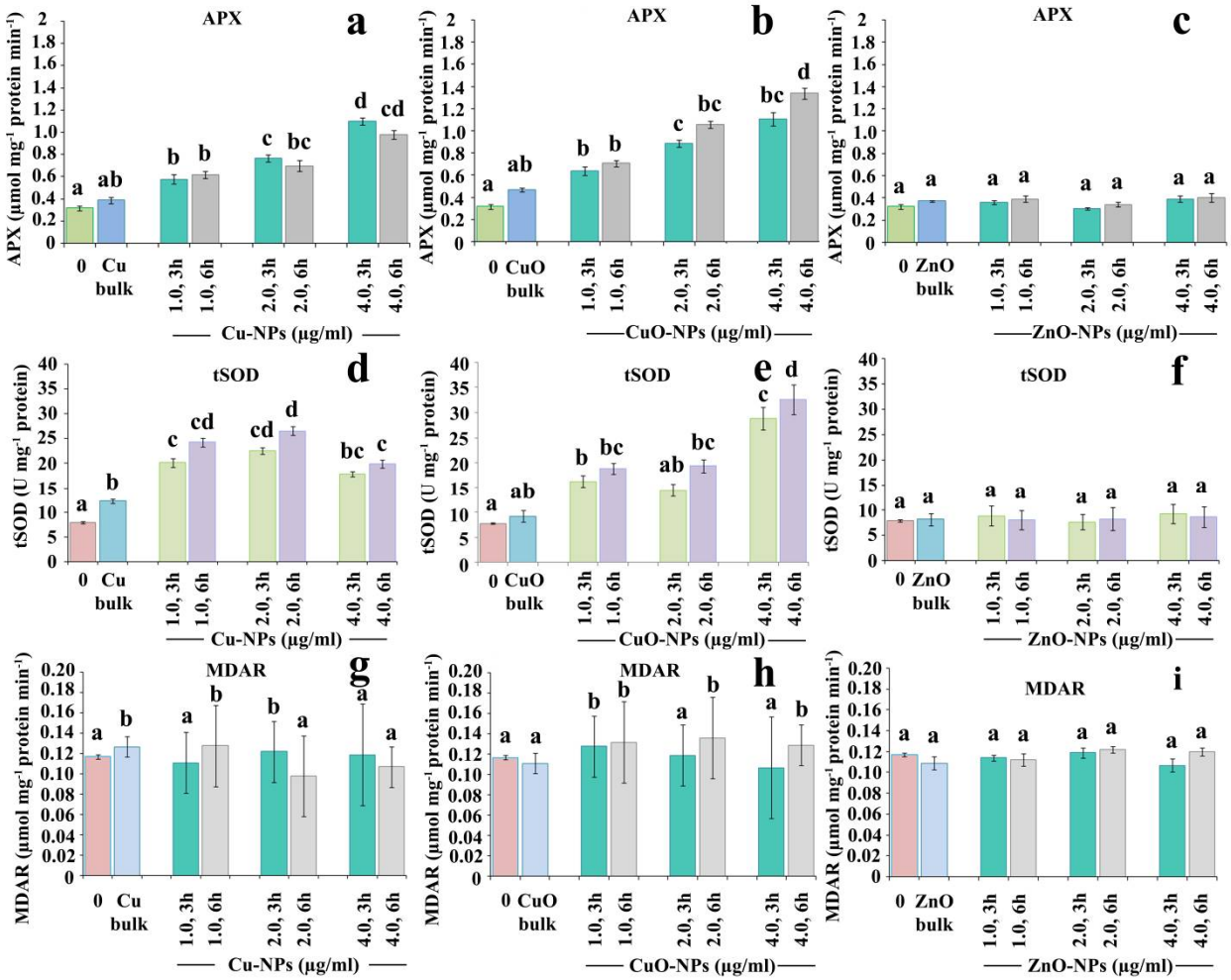

Figure 2 Antioxidant enzymes activity (APX: **a–c,** tSOD: **d–f,** MDAR: **g–i)** in host (*S. indicum).*

property in all the treated NPs, 5) non-synchronous enzymes response against Cu- and CuO-NPs treatments suggests

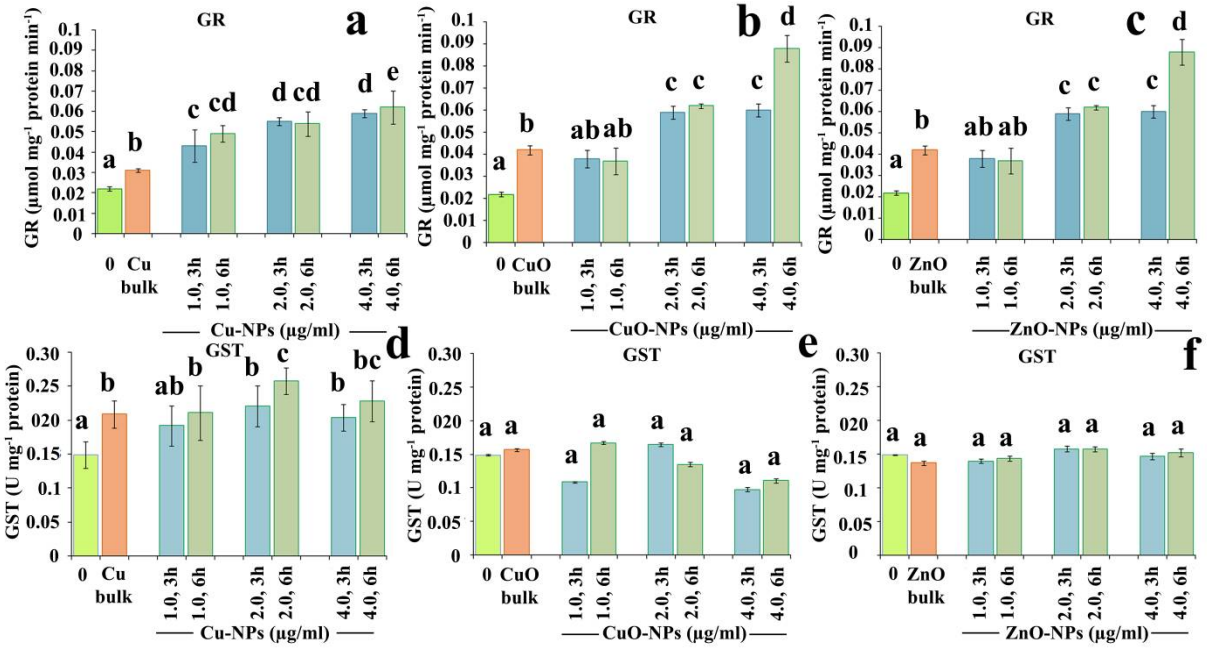

Figure 3 GR **(a–c)** and GST **(d–f)** enzyme kinetics in host.

Enhancement in APX reactivity indicates successful activation of initial step of acsorbate pool recycling [18]. Similarly, tSOD activity also enhances in Cu- and CuO-NPs treatments (Fig 2 d–f) and the elevation is mostly dose dependent (excepting 4.0 µg ml^{-1}, 3 and 6 h Cu-NPs and 2.0 µg ml^{-1}, 3h CuO-NPs). Such results highlight secondary defense response against reactive oxygen species [19]. At comparable doses, CuO-NPs also exhibit higher degree of tSOD reactivity than Cu-NPs.

Cellular level MDAR quantification reveals ground level enzyme reactivity in both Cu- and CuO-NPs in comparison to controls (Fig 2 g–i). Dose dependent significant ($p<0.05$) increase in GR activity is studied in both Cu- and CuO-NPs (excepting 1.0 µg ml^{-1}, 3 and 6h CuO-NPs); while, GST effectivity is insignificant excepting 2.0 µg ml^{-1}, 6h Cu-NPs (Fig 3 a–f). APX is one of the essential anti-oxidant enzyme responsible for conversion of H_2O_2 to H_2O [20] resulting in elevated production of monodehydroascorbate which needs to be recycled back to ascorbate by MDAR [21] to maintain the ascorbate pool for proper functioning of APX-MDAR complex in minimizing oxidative stress. However, result shows non-synchrony in APX-MDAR-GR-GST activity leading to malfunctioning of ascorbate-glutathione mediated oxidative stress reduction system. In the present investigation defense enzyme reactivity corroborates with NPs uptake measured in seedling ash which is possibly related to the size of the synthesized NPs.

Assessment of antioxidant enzyme responsiveness in the studied host highlights the following: 1) Cu-doped ZnO-NPs treatments show mostly insignificant antioxidant enzymes responses in relation to controls, 2) elevation in subcellular tSOD concentration in Cu- and CuO-NPs treatments demonstrate activation of secondary defense response, 3) enhance APX-GR reactivity exhibits successful functioning of initial stress minimization reaction associated with ascorbate pool recycling, 4) MDAR and GST shows base level kinetic prevalence of oxidative stress and 6) the antioxidant enzyme kinetics corroborates with NPs uptake.

CONCLUSION

Results demonstrate that Cu- and CuO-NPs exhibit non-synchronous activation of ascorbate pool recycling enzymes system along with successful functioning of secondary defense responses associated with tSOD activity and prevalence of oxidative stress.

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Conflict of Interest: The authors declare no conflict of interest**.**

References

1. Biener J., Wittstock A., Baumann T.F., Weissmüller J., Bäumer M. and Hamza A.V. 2009. Surface Chemistry in Nanoscale Materials. *Materials* 2: 2404–2428.
2. Braakhuis H.M., Park M.V.D.Z., Gosens I., De Jong W.H. and Cassee F.R. 2014. Physicochemical characteristics of nanomaterials that affect pulmonary inflammation. *Particle Fibre Toxicol.* 11: 18.
3. Ude V.C., Brown D.M., Viale L., Kanase N., Stone V. and Johnston H.J. 2017. Impact of copper oxide nanomaterials on differentiated and undifferentiated Caco-2 intestinal epithelial cells; assessment of cytotoxicity, barrier integrity, cytokine production and nanomaterial penetration. *Particle Fibre Toxicol*. 14: 31.
4. Ghosh M., Bandyopadhyay M. and Mukherjee A. 2010. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: Plant and human lymphocytes. *Chemosphere* 81: 1253-1262.

5. Saddick S., Afifi M. and Abu Zinada O.A. 2015. Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of Oreochromis niloticus and Tilapia zillii. *Saudi J. Biol. Sci.* http://dx.doi.org/10.1016/j.sjbs.2015.10.021.
6. Das D., Datta A.K., Kumbhakar D.V., Ghosh B., Pramanik A., Gupta S. and Mandal A. 2017. Assessment of photocatalytic potentiality and determination of ecotoxicity (using plant model for better environmental applicability) of synthesised copper, copper oxide and copper-doped zinc oxide nanoparticles. PLoS ONE 12: e0182823.
7. Nakano Y. and Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidise in spinach chloroplasts. *Plant Cell Physiol*. 22: 867-880.
8. Beyer W.F. and Fridovich I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.* 161: 559-566.
9. Miyake C. and Asada K. 1992. Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant Cell Physiol.* 33: 541-553.
10. Carlberg I. and Mannervik B. 1985. Glutathione reductase assay. Methods in Enzymol Academic Press, Orlando FL, vol. 113:pp. 484-495.
11. Aebi H. 1983. Catalase. In: Bergmeyer, H.U. Methods in Enzymatic Analysis, second English ed. Academic Press, New York, pp. 276-286.
12. Schwab F., Zhai G., Kern M., Turner, A., Schnoor, J.L. and Wiesner, M.R. 2015. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants--Critical review. *Nanotoxicol.* 10: 257-278.
13. Siddiqi K.S. and Husen A. 2016. Engineered gold nanoparticles and plant adaptation potential. *Nanoscale Res. Lett.* 11: 400.
14. Singh J., Michel D., Chitanda J.M., Verrall R.E. and Badea I. 2012. Evaluation of cellular uptake and intracellular trafficking as determining factors of gene expression for amino acid-substituted gemini surfactant-based DNA nanoparticles. J. *Nanobiotechnol.* 10: 7.
15. Firdessa R., Oelschlaeger T.A. and Moll H. 2014. Identification of multiple cellular uptake pathways of polystyrene nanoparticles and factors affecting the uptake: Relevance for drug delivery systems. *European J. Cell Biol.* 93: 323-337.
16. Kettler K., Veltman K., van de Meent D., van Wezel A. and Hendriks A.J. 2014. Cellular uptake of nanoparticles as determined by particle properties, experimental conditions, and cell type. *Environ. Toxicol. Chem.* 33: 481-492.
17. Kohan-Baghkheirati E. and Geisler-Lee J. 2015. Gene Expression, Protein Function and Pathways of Arabidopsis thaliana Responding to Silver Nanoparticles in Comparison to Silver Ions, Cold, Salt, Drought, and Heat. *Nanomaterials* 5: 436-467.
18. Massot C., Bancel D., Lopez Lauri F., Truffault V., Baldet P. and Gautier H. 2013. High Temperature Inhibits Ascorbate Recycling and Light Stimulation of the Ascorbate Pool in Tomato despite Increased Expression of Biosynthesis Genes. PLOS ONE 8: e84474.
19. Ishiga Y., Uppalapati S.R., Ishiga T., Elavarthi S., Martin B. and Bender C.L. 2009.The phytotoxin coronatine induces light-dependent reactive oxygen species in tomato seedlings. *New Phytol.* 181: 147-160.
20. Sharma P., Jha A.M., Dubey R.S. and Pessarakli M. 2012.Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot. Article* ID 217037.
21. Eastmond J. 2007. MONODEHYROASCORBATE REDUCTASE4 Is Required for Seed Storage Oil Hydrolysis and Post germinative Growth in Arabidopsis. *The Plant Cell* 19: 1376-1387.

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