Plant response to metal-containing engineered nanomaterials: an omics-based perspective

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Plant Response to Metal-Containing Engineered Nanomaterials: An Omics-Based Perspective

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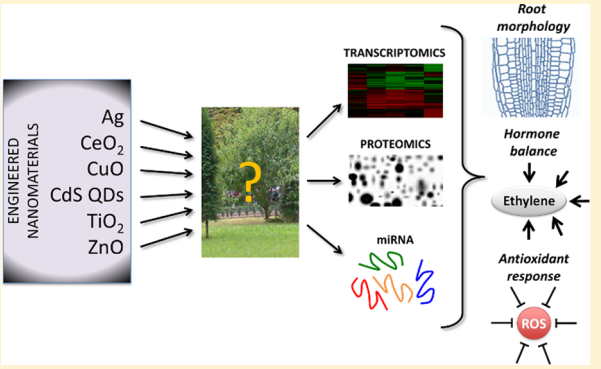
Supporting Information

ABSTRACT: The increasing use of engineered nanomaterials (ENMs) raises questions regarding their environmental impact. Improving the level of understanding of the genetic and molecular basis of the response to ENM exposure in biota is necessary to accurately assess the true risk to sensitive receptors. The aim of this Review is to compare the plant response to several metal-based ENMs widely used, such as quantum dots, metal oxides, and silver nanoparticles (NPs), integrating available "omics" data (transcriptomics, miRNAs, and proteomics). Although there is evidence that ENMs can release their metal components into the environment, the mechanistic basis of both ENM toxicity and tolerance is often distinct from that of metal ions and bulk materials. We show that the mechanisms of plant defense against ENM stress include the modification of root architecture, involvement of specific phytohormone signaling pathways, and activation of antioxidant mechanisms. A critical meta-analysis allowed us to identify relevant genes, miRNAs, and proteins involved in the response to ENMs and will further allow a mechanistic understanding of plant−ENM interactions.



INTRODUCTION

Engineered nanomaterials (ENMs), a class of materials with dimensions between 1 and 100 nm, are characterized by unique physicochemical properties that differ from their respective bulk materials.[1] The differences are a consequence of both their large surface area to mass ratio but also reflect the nature of the surface coating used, solubility, shape and morphology, and tendency toward self-aggregation.[2] In recent years, there has been a considerable increase in metal-based ENM production and marketing.[3] The global production of ENMs is forecast to be higher than 0.5 Mt by 2020;[4,5] meanwhile, concerns are being voiced over the environmental consequences of this level of production and release. There is an urgent need to gain better understanding of ENM properties and to assess their potential risks for human health and environment.[6−8] The interaction of ENMs with plants is particularly important, given that plants are the primary trophic level in several ecosystems and represent the base of the food chain for many animals, including humans.[3,4]

Plant response to ENM exposure is variable, depending significantly on factors, such as particle size and characteristics, dose, duration of exposure, plant species, and environmental conditions[9] (Table 1). Metal-based ENMs can be taken up by the plant roots either apoplastically or symplastically through the leaf cuticle, stomatal pores, or cuticle-free flowers.[3] The tendency of ENMs to cross the root barrier and translocate through the vascular system into various tissues is strongly affected by their physicochemical characteristics as well as by the plant species and rate of transpiration.[4,10−28] Cell wall composition, the presence of mucilage and other exudates, root symbiont activity, and the availability of soil organic matter all impact the mobility, bioavailability, and reactivity of ENMs.[13,29−33]

Negative effects of exposure to metal-based ENMs on germination, root and shoot growth, and on the number of leaves formed have been observed in *Arabidopsis thaliana* (L.) Heynh as well as in a number of crop species.[10,17,21,26,27,30,34−45] Although examples have been provided for the release of metal cations from ENMs, in general, free ions

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Table 1. List of Omics Studies Considered in This Review

paper	plant	plant organ	age of plants at treatment	ENM incubation time for omics analysis	dose	ENMs	particle size (nm)	ENM treatment effect	additional information[a]
				transcriptomic data					
García-Sánchez et al., 2015	*A. thaliana Col-0*	whole plant	3 weeks	2 days	0.2 mg L^{-1}	Ag NPs	10, 20, 40, and 80	no effect	a
Kaveh et al., 2013	*A. thaliana Col-0*	whole plant	seeds	10 days	5 mg L^{-1}	PVP–Ag NPs	20	negative effect	b
García-Sánchez et al., 2015	*A. thaliana Col-0*	whole plant	3 weeks	2 days	20 mg L^{-1}	TiO_2 NPs	10, 20, and 40	no effect	a
Landa et al., 2012	*A. thaliana Columbia*	roots	6 weeks	7 days	100 mg L^{-1}	TiO_2 NPs	<150	no effect	c
Tumburu et al., 2015	*A. thaliana Col-0*	whole plant	seeds	12 days	500 mg L^{-1}	TiO_2 NPs	33	positive effect	c
Tumburu et al., 2017	*A. thaliana Col-0*	roots and shoots	seeds	29 days	500 mg L^{-1}	TiO_2 NPs	33	no effect	a
Tumburu et al., 2015	*A. thaliana Col-0*	whole plant	seeds	12 days	500 mg L^{-1}	CeO_2 NPs	21	positive effect	c
Tumburu et al., 2017	*A. thaliana Col-0*	roots and shoots	seeds	29 days	500 mg L^{-1}	CeO_2 NPs	21	positive effect	a
Landa et al., 2012	*A. thaliana Columbia*	roots	6 weeks	7 days	100 mg L^{-1}	ZnO NPs	<100	negative effect	b
Landa et al., 2015	*A. thaliana Col-0*	roots	4 weeks	7 days	4 mg L^{-1}	ZnO NPs	20	negative effect	b
Tang et al., 2016	*A. thaliana Bay-0*	roots	15 days	2 h	10 mg L^{-1}	CuO NPs	30–50	negative effect	c
Marmiroli et al., 2014	*A. thaliana Ler-0*	whole plant	2 weeks	21 days	40 or 80 mg L^{-1}	CdS QDs	5	negative effect	a
				microRNA profiling					
Burklew et al., 2012	*N. tabacum*	whole plant	seeds	3 weeks	0.1, 0.5, or 1.0%	Al_2O_3 NPs	not indicated	negative effect	
Frazier et al., 2014	*N. tabacum*	whole plant	seeds	3 weeks	0.1 or 1.0%	TiO_2 NPs	<25	negative effect	
				proteomic data					
Vannini et al., 2013	*E. vesicaria*	roots and shoots	seeds	5 days	10 mg L^{-1}	PVP–Ag NPs	10	positive effect	
Mirzajani et al., 2014	*O. sativa*	whole plant	10 days	20 days	30 or 60 mg L^{-1}	Ag NPs	18.3	negative effect	
Vannini et al., 2014	*T. aestivum*	roots and shoots	seeds	5 days	10 mg L^{-1}	PVP–Ag NPs	10	negative effect	
Majumdar et al., 2015	*P. vulgaris*	seeds	seeds	102 days	62.5, 125, 250, or 500 mg/kg	CeO_2 NPs	8	no effect	

[a]Transcriptomic data considered in this review are characterized by whole database (a) or modulated gene set (b, $-1 < \log_2$ fold change < 1; c, $-2 < \log_2$ fold change < 2).

contribute only partially to the toxicity of many metal-based ENMs.[24,27,35,46] Several mechanisms have been proposed to explain the phytotoxicity of these materials.[3] Uptake of ENMs into the root may lead to the blocking of root pores, effectively inhibiting the apoplastic flow of water and micronutrients.[24,47,48] The induction of reactive oxygen species (ROS) is a commonly observed consequence of the exposure to metal-based ENMs and significantly contributes to the observed toxicity.[39,49−53] ROS induce lipid peroxidation, alter plant cell membranes and wall structures,[54] and directly damage proteins and DNA.[3] Many ENMs cause genotoxic effects, including chromosomal aberrations, mitotic division impairment, and cellular disintegration.[21,39,49,50,55]

Notably, there are reports in the literature showing that ENM exposure can also positively influence plant growth and development.[22,56−61] For example, in tomatoes (*Solanum lycopersicum* L.), CeO_2 nanoparticles (NPs) slightly improve plant biomass, although the data suggest that the second generation of seedlings show some physiological deficits as compared to those of control plants.[22,62]

Although a number of reviews on plant−ENM interactions have been published,[3,44,45,53,63−69] the specific purpose of this work is to provide a comprehensive evaluation and integration of omics data describing the complex molecular networks in ENM response. High-throughput data considered in this Review (Table 1) include the transcriptomic response of *A. thaliana* to Ag,[70,71] TiO_2,[61,70,72,73] CeO_2,[61,73] ZnO,[72,74] and CuO[75] NPs or cadmium sulfide (CdS) QDs[46] (Table S1). miRNA profiling data are obtained from tobacco (*Nicotiana tabacum* L.) plants exposed to TiO_2[76] and aluminum oxide (Al_2O_3)[77] NPs (Table 1), while the proteomic data sets involve the response to Ag NP exposure in rocket salad (*Eruca vesicaria* L. Cav.),[78] rice (*Oryza sativa* L.)[79] or wheat (*Triticum aestivum* L.),[80] and the response to CeO_2 NPs in kidney beans (*Phaseolus vulgaris* L.)[81] (Table S2). A systems biology approach integrating data from large-scale measurements can lead to a more mechanistic understanding of the plant

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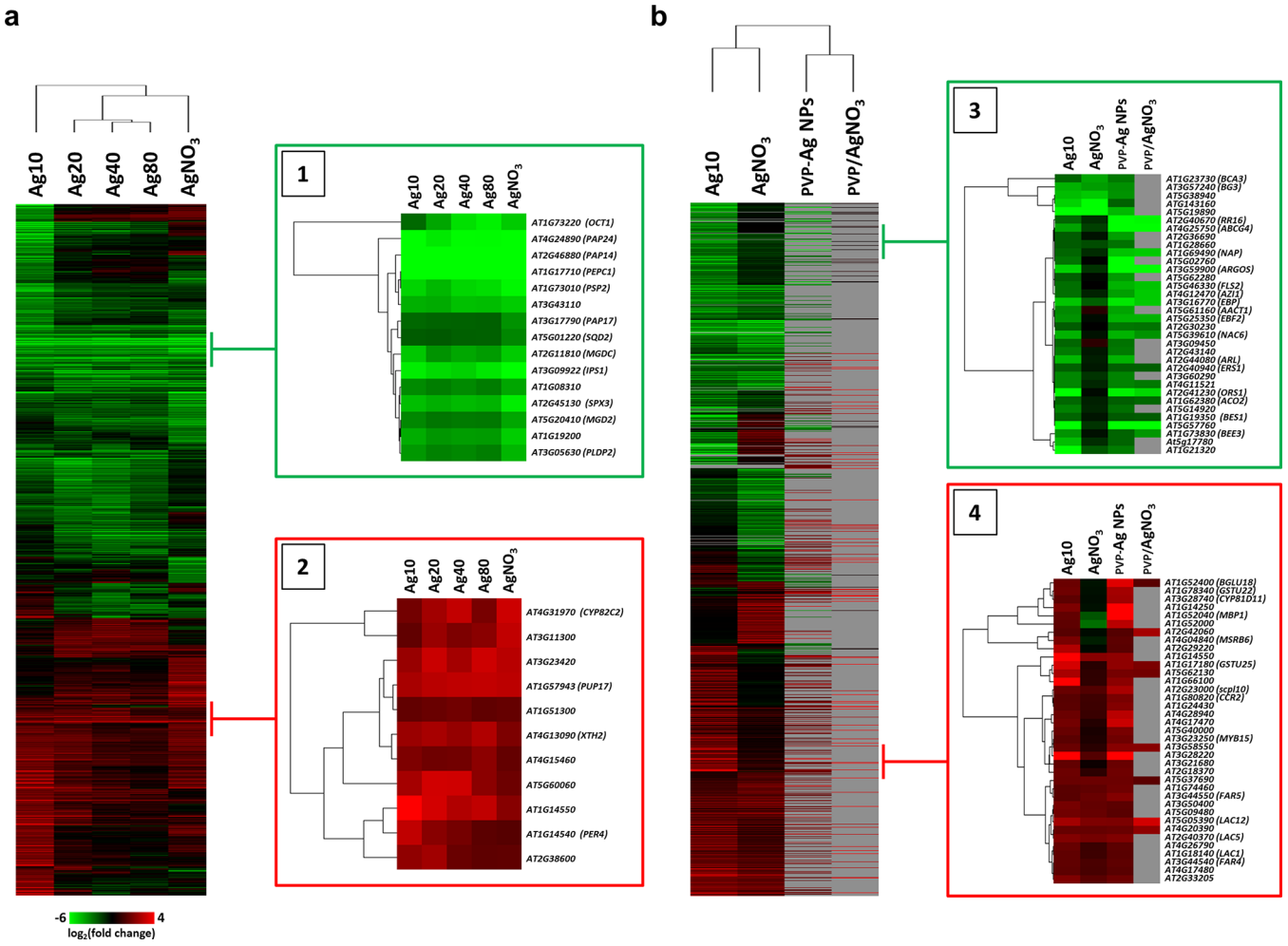

Figure 1. Hierarchical clustering of Ag NP-responsive genes in *A. thaliana*. (a) Genes altered with respect to their transcription level following a 2 day exposure to Ag NPs of 10, 20, 40, or 80 nm diameter (respectively, Ag10, Ag20, Ag40, and Ag80) and bulk material ($AgNO_3$). The heat map in box 1 (green) displays genes responding to Pi starvation that are down-regulated by all levels of Ag stress. Box 2 (red) shows genes up-regulated by both Ag NPs and bulk treatments. These include *At4g13090* (Xth2, xyloglucan/xyloglucosyl transferase), *At4g31970* (Cyp82c2, cytochrome P450), *At1g57943* (Atpup17, purine transporter), *At1g14540* (Per4, peroxidase involved in the response to oxidative stress) and *At1g14550* (putative anionic peroxidase). (b) Genes altered with respect to transcription level following exposure to either Ag10, PVP-Ag NPs, bulk material, or Ag^+ ions in the presence of the stabilizing polymer PVP (PVP–$AgNO_3$).[71] The heat map in box 3 (green) displays transcripts down-regulated in all NP-exposed plants, including *At2g40940* (Ethylene Response Sensor 1, Ers1) and *At5g61160* (anthocyanin 5-aromatic acyltransferase 1, Aact1). Genes up-regulated in plants exposed to Ag NPs are shown in box 4 (red), including *At1g18140* (laccase 1, Lac1), *At5g05390* (laccase 12, Lac12), *At2g40370* (laccase 5, Lac5), and *At3g28740* (cytochrome P450, Cyp81d11).

physiological response to ENM exposure and a more-accurate assessment of risk.[82−85]

METHODOLOGICAL NOTES ON COMPARATIVE IN SILICO ANALYSIS OF OMICS DATA

In this Review, we summarize a number of multiomics studies on plant response to ENM stress (Tables 1, S1, and S2). ENM dose and particle size, as well as germination conditions and developmental stages assayed in the experiments, are annotated (Table 1).

For microarray data, relative expression ratios (treatment over control) are $\log_2$-transformed, and genes showing expression ratios ≥ 2 or ≤ 0.5 are classified as up- or down-regulated by ENM treatment. Gene ontology (GO) analysis is conducted using the Plant GeneSet Enrichment Analysis toolkit.[52] Biological processes associated with ENM toxicity-modulating genes are identified and evaluated for statistical significance (P value of $\leq 1 \times 10^{-03}$). A hierarchical clustering analysis (Pearson correlation, average linkage) of differentially expressed transcripts is achieved using Cluster v3.0 software,[86] and the clustered data are visualized using Java Treeview.[87] MapMan v3.6.0RC1[88] is employed to map transcriptomic data to metabolic pathways and other biological processes.

Box plots (Figure S1) and principal component analysis (PCA), performed with R software (https://www.r-project.org/) are used to show the distribution of gene expression data and extract major variables (in the form of components) from the large set of variables available in the transcriptomic data set (Table S1). The EnrichmentMap plug-in[89] is used to visualize as a network the results of an analysis performed with the DAVID Functional Annotation Tool[90] using the Cytoscape network visualization software.[91]

SILVER NANOPARTICLES

Transcriptomic Response. A pair of studies have investigated the transcriptional response of *Arabidopsis* exposed

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to Ag NPs (nanosilver) using whole-genome expression microarrays (Table S1): García-Sánchez et al.[70] reported that a brief exposure to low doses of 10−80 nm nanosilver did not affect the plant growth; Kaveh et al.[71] reported moderate toxicity to 10 day old seedlings exposed to 20 nm nanosilver in the presence of the stabilizing polymer polyvinylpyrrolidone (PVP).[92] A significant overlap is observed between the sets of genes differentially expressed in response to nanosilver[70,71] and bulk material[70] or Ag^+ ion[71] treatments (Figure 1), but the transcriptomic response induced by a brief exposure to the smaller 10 nm Ag NPs differs to a greater extent from the bulk treatment (Figure 1a). Notably, several studies suggested that ENM uptake and toxicity increased with decreasing particle size.[14,34,93−95]

A Gene Ontology (GO) enrichment analysis (Table S3) reveals that gene expression changes induced by a brief exposure to smaller nanosilver (10 nm diameter) are different from those by the PVP−Ag NPs (Figure 1b). Genes encoding for proteins involved in response to ROS (e.g., peroxidases; superoxide dismutases, SODs) and in xylem development were repressed by an early exposure to nanosilver but induced by PVP−Ag NPs (Table S3). Early transcriptional repression of genes encoding for antioxidant enzymes upon exposure to nanosilver can be explained considering the central role that ROS have in ENM stress response.[3,53] In fact, ROS are essential components of signal transduction in response to developmental and environmental cues and transcriptional regulatory networks can be activated upon long-term nanosilver treatment to maintain nontoxic levels of ROS.[96]

A brief exposure to nanosilver (10−80 nm diameter) also down-regulates genes involved in root development (Tables S1 and S3). An altered root morphology has been identified as a consequence of exposure to various ENMs;[76,79,93,97] nanosilver appears to inhibit primary root growth by acting directly on the root tip meristems[76,79,93,94,97] and on root-hair growth.[43,70] Genes implicated in differentiation of trichoblasts, specialized epidermal cells from which root hair emerge, as well as genes responsive to ethylene and auxin, positive regulators of root hair development,[98] are indeed down-regulated by an early exposure to nanosilver (Table S3), indicating that plants can respond quickly to nanosilver by reducing the root hair growth. In fact, a hairless-like root phenotype was noted in *A. thaliana* plants upon nanosilver treatment.[70] Root-hair function is related to absorption of water and nutrients, and a long-term repression of root hair development due to nanosilver exposure could have negative effects on plant growth and yield.[64,99] Ag^+ ions may occupy the ethylene-binding pocket of the ETR1 receptor and prevent downstream hormone signaling necessary for the root hair development.[98] It is possible that nanosilver or, more likely, the released Ag^+ ions can inhibit the ETR1-dependent ethylene signaling pathway.

Adaptive changes in root architecture may be mediated by ethylene and auxin in response to low phosphorus (Pi) concentrations, a condition that promotes lateral and hairy root formation but suppresses primary root growth.[100] Interestingly, genes induced in the response to Pi starvation are repressed by an early treatment to both nanosilver and the bulk material (Table S3). In addition, genes involved in galactolipid biosynthesis (*MGD2*, *MGD3*, and *SRG3*) are also significantly down-regulated by both forms of Ag (Table S3). Membrane phospholipids, which constitute ∼30% of total phosphorus storage in the plant,[101] are hydrolyzed in the response to Pi starvation and replaced by nonphosphorus lipids, such as galactolipids, which serve to maintain the functionality and structure of plasma membranes.[102] Nanosilver exposure can likely trigger alterations in several pathways involved in an efficient mobilization and acquisition of Pi from the growth medium and intracellular stores, impairing membrane phospholipid composition as well as root development. Consequently, nanosilver may have negative effects on plant growth under Pi-deficient conditions.

The early transcriptional response to nanosilver (10−80 nm diameter) also prompted repression of pathogen-activated genes involved in the systemic acquired response (SAR) mediated by salicylic acid (SA) as well as genes involved in abiotic stress responses. Geisler-Lee et al.[41] showed that exposure to nanosilver compromises plant ability to limit pathogen growth. Nanosilver exposure of infected plants was associated with increased bacterial colonization, but supplementation with SA prior the addition of ENMs prevents bacterial growth and also counteracts the inhibition of root hair formation caused by ENM stress.[70] A repression of SAR genes under periods of prolonged ENM exposure may therefore negatively affect the plant capacity to tolerate biotic stress.

Genes strongly up-regulated upon early and long-term nanosilver treatments encode for proteins involved in defense response (Table S1): defensin-like proteins, plant thionin, β-glucosidases, cytochrome P450 proteins, and τ-class glutathione S-transferase (GST) members. GST expression is induced by a wide variety of stress conditions,[103] including ENMs,[104,105] and the over-expression of GST isoforms after nanosilver exposure might be needed for the detoxification of released Ag^+ ions by binding to thiol groups of glutathione (GSH) mediated by these enzymes.[104] PVP−Ag NP treatment also induces the transcription of a small operon-like cluster of genes, which are required for the synthesis and modification of the triterpene thalianol (Table S3), a class of secondary metabolites frequently implicated in plant defense response.[106]

In addition, genes involved in phenylpropanoid synthesis, in particular suberin, are significantly up-regulated upon nanosilver exposure but not in response to Ag^+ ions (Table S3). Phenylpropanoids are precursors of diverse secondary metabolites, such as lignins, suberin, and flavonoids, and can play important roles in plant development and stress response.[107,108] Suberin is a cell-wall polymer composed predominantly of long-chain hydroxylated fatty acids and is deposited apoplastically to generate a lipophilic barrier to the uncontrolled flow of water, gases, and ions;[109] thus, suberin provides a first line of defense against abiotic stresses, such as ENM treatment.

Long-term exposure to PVP−Ag NPs also up-regulates a number of genes required for the synthesis of cell-wall polysaccharides and lignin (Table S3); these biomolecules play a key role in modulating cell-wall structure in response to several stressors.[110] Lignin deposition, which occurs late in xylem cell differentiation, serves to waterproof the cell wall;[111] therefore, a prolonged exposure to nanosilver could lead to a decrease in cell wall extensibility and/or turgor. Laccases are responsible for the extracellular polymerization of lignin precursors,[112] and the genes encoding these enzymes are also up-regulated by PVP−Ag NP exposure (Table S1).

Proteomic Response. A total of three studies of plant proteomic response to nanosilver exposure have been published to date, involving *E. vesicaria*,[78] rice,[79] and wheat[80] (Tables 1 and S2). PVP−Ag NP (10 nm diameter) treatment did not show any significant effect on *E. vesicaria* seed germination, whereas an increased root growth was noted.[78] Proteomic

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analysis shows only a limited overlap between the response to PVP−Ag NPs and bulk material (Table S2). Both forms of Ag strongly induce accumulation of proteins related to oxidative stress response (SOD, peroxiredoxin) and the seed-specific proteins belonging to the jacalin lectin family,[113] which catalyze the hydrolysis of glucosinolates, a group of S-rich metabolites.[114] Glucosinolates may be considered a potential storage form of sulfur and an increased hydrolysis of these metabolites has been reported under S deficiency.[114] In accordance with these observations, the levels of key enzymes in cysteine and methionine synthesis are enhanced by ENM-induced stress, indicating that the S metabolism can play a crucial role in nanosilver tolerance. Interestingly, thiol ligands, such as cysteine, strongly bind Ag^+ ions leading to increased dissolution rate of nanosilver.[115]

Synthesis of seed storage proteins, as cruciferins, is increased by nanosilver treatment. In *Arabidopsis*, seedling germination requires the breakdown of cruciferins, which are used as an initial source of nitrogen.[116] Such a mechanism could be correlated with the positive effects induced by ENM treatment in rocket root growth.

Proteomic analysis also showed an increase in the levels of detoxifying enzymes (e.g., glucosidase 23)[117] localized in endoplasmic reticulum (ER) in *E. vesicaria* plants exposed to nanosilver. An altered ER morphology is observed upon nanosilver treatment, and these results indicate that ER might be a crucial cellular target of the plant response to PVP−Ag NPs.[78,80] In addition, nanosilver exposure decreases the abundance of two vacuolar-type proton ATPase subunits (Table S2), suggesting a role for the vacuole in ENM detoxification, as reported in other species.[38,93]

Mirzajani et al.[79] reported protein expression changes in rice roots exposed to nanosilver (18 nm diameter; Tables 1 and S2). Nanosilver treatment in *O. sativa* enhances the cellular levels of proteasome subunits and a 60S acidic ribosomal protein, indicating that the accumulation of damaged proteins, followed by their degradation via the ubiquitin pathway, and de novo protein synthesis are processes associated with ENM stress response. As in *E. vesicaria*,[78] the levels of enzymes involved in oxidative stress response (e.g., SOD and ascorbate peroxidase) are increased in rice plants treated with nanosilver. This could be the consequence of an enhanced transcription of these genes, as observed by Ag NP treatment in *Arabidopsis* (Table S1).[43,71] Moreover, nanosilver exposure in rice reduces the abundance of Ca^{2+}-binding messengers calmodulin 1 and 3, known to be involved in signal transduction in response to various biotic and abiotic stressors;[118,119] an alteration of the Ca^{2+}-signaling pathway mediated by nanosilver can negatively affect cell metabolism in rice.

Proteomic analysis was also conducted in wheat treated with 10 mg L^{-1} PVP-Ag NPs (10 nm diameter), a level sufficient to compromise both root and shoot elongation (Tables 1 and S2).[80] PVP-Ag NP treatment enhances the accumulation of three α-amylases in wheat roots, and increased levels of these proteins can be related to the observed reduction of starch grains in treated roots.[80]

In both rocket and wheat,[78,80] PVP−Ag NP exposure results in an increase in the levels of malate dehydrogenase (MDH), an enzyme which catalyzes the reversible reaction of oxaloacetate to malate. A higher root exudation of organic acids, such as malate, mediated by MDH is known to be connected with metal stress tolerance.[120] Organic acids in root exudates can play a dual role in ENM mobility and bioavailability: they could either mobilize ENMs to accelerate uptake in plants or complex with ENMs to inhibit their translocation.[121] Proteins belonging to the 14−3−3 family, known to stimulate the activity of the plasma membrane H^+-ATPase and increase root exudation,[122] are also accumulated in root cells when exposed to PVP−Ag NPs (Table S2).

As observed in rocket and rice,[78,79] nanosilver exposure also affects the concentration of proteins with a role in plant defense, such as GSTs, peroxidases, or chitinases.[123,124] In addition, PVP−Ag NP exposure enhances the levels of energetic metabolism enzymes (Table S2), and this likely reflects an increased energy demand during nanosilver stress. Higher levels of the eukaryotic translation initiation factor 5A2 (eIF5A), the 60S acidic ribosomal protein but also of proteolytic enzymes suggest that nanosilver may affect protein synthesis and degradation in wheat, as reported in rice.[79] Although differences in the time of exposure, dose, particle size, and plant material can make it difficult to obtain a mechanistic understanding of plant response to nanosilver, different omics data show that nanosilver exposure triggers plant defense pathways, involving the antioxidant response or synthesis of sulfhydryl-containing ligands.

TITANIUM DIOXIDE NANOPARTICLES

Transcriptomic Response. Several reports have been published[61,70,72,73] in which *Arabidopsis* was exposed to uncoated TiO_2 NPs (nanotitania), with experiments differing in particle size, concentration, time of exposure, and plant developmental stage (Table 1). Analysis of differentially expressed genes reveals a general down-regulation induced by early exposure (2 days) to nanotitania (10−40 nm diameter; Tables 1 and S1 and Figure S2).[70] Conversely, a more prolonged exposure (29 days) to high concentrations (500 mg L^{-1}) of nano titania (33 nm diameter) up-regulates 55% and 63% of transcripts in roots and shoots of *Arabidopsis* seedlings, respectively.[73] Smaller changes in gene expression (Figure S2) are instead produced in *Arabidopsis* by nanotitania treatments for 7 to 12 days.[61,72] Hierarchical clustering analysis reveals that the transcriptional profiles depend more strongly on the time of exposure (or on plant materials) than on the size or doses of the ENMs, and that response to short-term exposure to nanotitania[70] is rather similar to that induced by bulk material (Figure S2).

Early exposure to nanotitania (10−40 nm; Tables 1 and S4) causes down-regulation of genes encoding proteins involved in pathways usually associated with plant stress responses, such as ROS detoxification (e.g., peroxidases), triterpenoid and phenylpropanoid metabolism, or with hormone-signaling pathways involved in the response to SA, jasmonic acid (JA), ethylene, and brassinosteroids (BRs). Similar to what was observed with nanosilver (see above), genes classified into these GO categories are significantly up-regulated during the longer-term exposure to nanotitania (33 nm)[73] (Table S4). Furthermore, SA supplement rescues the depressive effects of nano titania on root-hair development, as observed for nanosilver.[70]

Plant response to water stress is mainly controlled by a complex molecular network regulated by abscisic acid (ABA) and the activities of transcription factors (TFs) involved in the regulation of stomatal responses to enable plants to adapt and survive.[125] Genes encoding components of ABA signaling pathway, involved in stomatal complex development, lignin biosynthesis, in response to chitin (e.g., chitinases) and to water

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deprivation (e.g., aquaporins) are significantly induced by long-term exposure to TiO_2 NPs[73] (Table S4). A prolonged treatment with nano titania could therefore induce drought stress. Nanotitania accumulation in maize (*Zea mays* L.) primary roots is, in fact, accompanied by a reduction in the cell-wall pore diameter that negatively affects water transport and transpiration.[47] In cucumber (*Cucumis sativus* L.), TiO_2 NPs are transported to the leaf trichomes, suggesting that these structures serve as a sink or even an excretory organ for these ENMs.[126] Trichomes, which are generally considered to have evolved to protect against water loss and herbivorous animals, are also involved in defense against heavy metal stress.[127]

Nanotitania treatment also induces genes associated with photosynthesis and chloroplast organization (Table S4). In *S. oleracea*, TiO_2 NPs increase light absorbance, chlorophyll formation, and plant photosynthetic rates.[57,60,128,129] These ENMs are thought to enter the chloroplast, where they likely promote energy transfer and oxygen evolution in photosystem components, thereby accelerating the photosynthetic reactions. It is also possible that nano titania can protect the chloroplast from excessive light by augmenting the activity of antioxidant enzymes.[57]

A high induction of genes in the GO category "microtubule organization" is also observed upon long-term exposure to nano titania (Table S4).[73] Small TiO_2 NPs (2.8 nm diameter) can induce microtubule disorganization in leaf epidermal and stomatal cells, followed by the 26S proteasome-dependent degradation of tubulin monomers.[130] This effect could be a secondary consequence of ROS generated by these ENMs[2] but could also arise from a direct physical interaction between the ENMs and the cytoskeleton. In fact, TiO_2 NP binding to microtubules has been observed in vitro, resulting in conformational changes to the cytoskeleton.[131]

Case of Post-Transcriptional Regulation: miRNA Response to TiO_2 NP Exposure. A study with tobacco[76] showed that nanotitania (25 nm diameter; Table 1) exposure inhibits root elongation and biomass formation and significantly influences the expression profiles of several microRNAs (miRNAs), short noncoding RNA (about 22 nucleotides in length) with a role in plant development and response to environmental stresses,[132,133] usually controlling mRNA stability or translation of target genes. Nano titania exposure strongly increases the expression levels of miR395 and miR399, and to a lesser extent, that of miR159, miR169, miR172, miR393, miR396, and miR398.[76] miR395 and miR399 control plant adaptive responses to nutrient stress.[134] miR395 expression is greatly increased under sulfate starvation, and its known targets are transcripts involved in sulfur assimilation;[135] these data are in agreement with the up-regulation of glucosinolate metabolism genes observed in *Arabidopsis* plants exposed to ENMs[61,73] and it is possible that symptoms of S starvation may be induced by nano titania exposure in tobacco. In *Arabidopsis*, miR399 is up-regulated by Pi deficiency,[136] and its mature form is translocated from shoot to root via the phloem, where it targets the transcript of the gene encoding E2-conjugase Pho2, leading to the expression of Pi transporters.[137]

The miR169 family is conserved in plant species and mediates the transcriptional regulation of several genes involved in plant development and in response to environmental stresses. The miR169 family responds differentially to nutrient deficiency in *Arabidopsis*;[133] nitrogen starvation up-regulates miR169d−g, while S and Pi starvation reduces the abundance of nearly all miR169 members.[136] The compromised growth and development of tobacco seedlings challenged with TiO_2 NPs[76] may therefore reflect a nutrient deficiency induced by ENM exposure. The overabundance of miR169a and miR169c reduces the transcriptional levels of *NFYA5*, encoding for a transcriptional regulator of drought tolerance.[138,139] Drought stress also enhances the abundance of miR159.[140] Thus, it is also possible that exposure to nanotitania causes water stress in tobacco, as has been shown for both maize[47] and *Arabidopsis*.[61,73] In tobacco, miR395, miR399, miR169, miR398, and miR159 are also induced when plants are exposed to Al_2O_3 NPs,[77] which have a negative effect on root growth and germination[26,34,77] (Table 1).

Both miR163[72] and miR408[70] are reduced in abundance when *Arabidopsis* is exposed to nanotitania. Targets of miR163 are genes for components of the defense pathways,[141] while those of miR408 encode various Cu-containing proteins, such as plantacyanin and laccases. Plantacyanin is essential for electron transfer between the cytochrome b6f complex (plastoquinol−plastocyanin reductase) and photosystem I.[142] Laccases are involved in different physiological mechanisms, such as in lignin synthesis, maintenance of cell wall structure and integrity[143] and response to stress.[136] It is relevant that genes encoding components involved in photosynthesis and lignin metabolic processes are up-regulated by nano titania in *Arabidopsis* (Table S4).

In summary, plant general stress response based on phenylpropanoid metabolism (e.g., lignin), hormone signaling pathways and ROS detoxification is involved in response to nanotitania and nanosilver. Transcriptomic profiling (including miRNA) analyses show that nutritional starvation and drought stress are closely associated with nano titania toxicity. These results are in agreement with those of two recent papers[144,145] focused on the metabolomic response of *O. sativa* plants treated with nanotitania. The studies show nanotitania exposure yields high levels of aspartic and glutamic acids, indicative of an increase in GSH metabolism and instrumental in maintaining the intracellular redox status,[144,145] and increased levels of linoleic and linolenic acid in treated rice leaves,[144] suggesting a potential membrane lipid peroxidation. High levels in plants of the multifunctional amino acid proline, which plays various roles in abiotic stress including drought,[146] are also observed in rice upon nanotitania treatment.[144,145]

CERIUM DIOXIDE NANOPARTICLES

Transcriptomic Response. A pair of reports that were recently published[61,73] (Table 1) show that CeO_2 NPs (nanoceria; 21 nm in diameter) promote seed germination and seedling growth in *Arabidopsis*. The up-regulation of genes (Table S5) involved in water and nutrient uptake, trichoblast differentiation, and lateral root and xyloglucan metabolism is in agreement with the observation that seedling growth was enhanced by nanoceria treatment.[61] Notably, xyloglucan catabolism increases cell wall extensibility[147,148] that, in association with an increased nitrate accumulation (Table S5),[149] can lead to growth stimulation.

As previously described for nanosilver and nanotitania, a prolonged exposure to nanoceria (29 days)[73] increases the transcription of genes repressed by ENM treatment performed for shorter times (12 days).[61] Genes associated with several stress responses, including ROS detoxification, various metabolic processes associated with SAR, response to ethylene stimulus and S-containing compound metabolism are repre-

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sentative of this differential molecular response associated with different times of ENM exposure. A strong down-regulation of genes involved in oxidative stress response has been observed after shorter time of nanoceria treatment[61] (Table S5), indicating that ROS may play a crucial role at early stages of *Arabidopsis* seed germination. Interestingly, ROS are produced during germination in radish through an active, developmentally controlled, physiological process for protecting the emerging seedling against pathogens and other stressors.[150]

Proteomic Response. Majumdar et al.[81] reported a proteomic analysis in kidney bean seeds exposed to nanoceria (8 nm diameter; Tables 1 and S2). The levels of 23 proteins are differentially modulated upon nanoceria exposure; the majority of these proteins (91%) are under-abundant in treated plants. Although the plants did not exhibit overt toxicity, the levels of seed proteins associated with nutrient storage (phaseolin), carbohydrate metabolism (lectins), and protein storage (legumin) were significantly reduced in a dose-dependent manner (Table S2). The authors suggest that nanoceria could impair the nutritional content and quality of kidney beans. Lectins, associated with carbohydrate metabolism, also play a role in defense against biotic stress.[151] Therefore, their reduction indicates that nanoceria could diminish pathogen resistance in beans. Increased levels of purple acid phosphatase suggest that nanoceria can induce better Pi acquisition, in agreement with a higher Pi content observed in plants exposed to these ENMs.[81,152]

Therefore, long-term treatment with nanoceria in *Arabidopsis* plants increases expression of genes associated with SAR, ethylene-dependent pathway, S-containing compound metabolism, and in the oxidative stress response.[73] In the same way, a recently published study on a proteomic and metabolomic analysis in *Phaseolus vulgaris* L.[153] shows that nanoceria alters the abundance of antioxidant compounds, such as carotenoids and phenolics, glucosinolate metabolism, and the abundance of some key enzymes involved in response to oxidative stress, such as ascorbate peroxidase and glutathione peroxidase. In the seeds of exposed kidney beans, many under-abundant proteins are involved in nutrient storage, carbohydrate metabolism, and protein storage.[81]

■ ZINC OXIDE NANOPARTICLES

ZnO NPs have been reported to be more toxic than other ENMs.[26,27,72,74] Different doses (4−100 mg L^{-1}) of ZnO NPs (20−100 nm diameter) negatively affect plant growth and morphology and induce similar transcriptional changes in *Arabidopsis* (Tables 1 and S6).[72,74] GO analysis of the affected genes (Table S6) revealed commonalities with the response to Zn^{2+} ions.[154] The up-regulation of genes (Table S1) encoding proteins involved in metal binding, transport (e.g., Nramp4, Zif1, Hma4), metal homeostasis and detoxification (e.g., metallothioneins and oligopeptide transporter Opt3) suggests that Zn^{2+} ion release by ZnO NPs is a key factor in mediating their toxicity.[74]

ZnO NP exposure strongly represses genes involved in the biosynthesis of BRs (Table S6), which have been shown to play a critical role in alleviating heavy metal stress.[155] BR supplementation to tomato seedlings treated with ZnO NPs reduces oxidative stress, by increasing the activities of key antioxidant enzymes, and decreases Zn content in plants.[155] Negative effects induced by ENM treatment in *Arabidopsis* can, therefore, be related to the repression of BR biosynthesis genes.[72,74]

ZnO NP exposure induces the expression of genes involved in N and Pi starvation and in lateral root formation, while it represses genes for primary root and root hair development (Table S6). *PHR1*, a master regulator of the plant transcriptional response to Pi starvation,[156] along with the transcription factor *WRKY75*, is induced by exposure to ZnO NPs[72] but not to Zn^{2+} ions (Table S1).

The presence of ZnO NPs reduces the abundance of transcripts involved in the modification and degradation of hemicellulose (Table S6), which is able to adsorb heavy metal ions.[45,157] ZnO NPs also induce alterations in cell division, cell structure, and nucleosome assembly, leading to perturbations in DNA packaging and transcriptional regulation (Table S6). As reported for other ENMs,[79,80] ZnO NP treatment inhibits ribosome biogenesis and, consequently, protein synthesis, increases protein degradation, and down-regulates transcripts involved in electron transport and energy production, especially photosynthesis (Figure S3 and Table S6). These adverse effects can be related to an increased production of ROS induced by these ENMs.[10]

In summary, negative effects are observed in the roots of *Arabidopsis* upon ZnO NP exposure. Response to ZnO NPs involves several pathways centered on oxidative stress response, root architecture remodeling, protein synthesis/turnover and energy balance. Modulation of key proteins and enzymes involved in metal homeostasis and detoxification indicate that Zn^{2+} ions are released by these ENMs. A gap in the current literature is the lack of proteomic studies focused on plant response to ZnO NPs; future research efforts should target pathways involved in the response to ZnO NPs so as to provide necessary mechanistic information for an accurate assessment of risk from these particles.

■ COPPER OXIDE NANOPARTICLES

Experiments performed by Tang et al.[75] on *Arabidopsis* seedlings exposed to CuO NPs (30−50 nm diameter; Table 1) under hydroponic conditions showed a reduction in root elongation. In these conditions, an altered expression of genes that are responsive to oxidative stress, phenylpropanoid biosysthesis and several hormone signaling pathways is observed (Table S7). Although there are no reports in literature of microarray experiments conducted in *Arabidopsis* plants treated with Cu^{2+} ions under experimental conditions comparable to those of Tang et al.[75] to use as a comparison, it is possible to hypothesize that metal ions can be released by these ENMs;[158] this aspect could partially explain some effects reported, as observed in *O. sativa* by Wang et al.[159] For example, CuO NPs strongly up-regulate *ZAT12*, encoding a transcription factor involved in abiotic stress response,[160] that play a key role in ROS signaling pathway.[75] Zat12 also seemed to be involved in response to metal ions (Cu^{2+} and Cd^{2+}) and iron deficiency.[161] Furthermore, *Zat12* is co-expressed with the gene *orf31*, a chloroplastic electron carrier involved in photosynthesis that has been identified as putative biomarker of ENM exposure and effect in some crops.[162,163] Wang et al.[164] reported how CuO NPs inhibited general chloroplast functionality, particularly through ROS generation and electron transport chain inhibition. Nair and Chung[165] reported primary root growth delay, enhanced lateral root formation, and loss of root gravitropism upon CuO NP exposure. As observed for ZnO NPs, the transcription factor WRKY75 involved in the transcriptional response to Pi starvation was also up-regulated by CuO NP treatment (Table S1).

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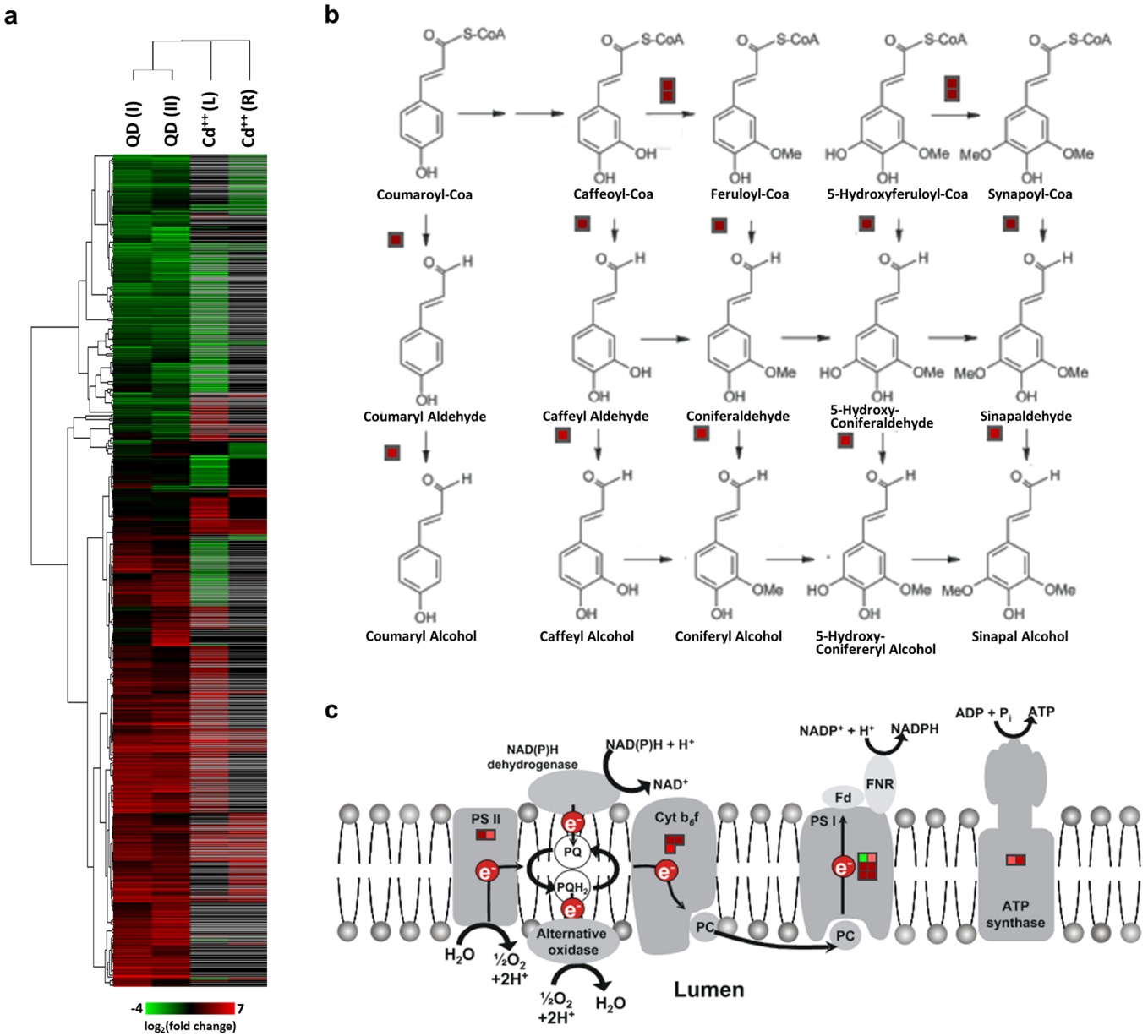

Figure 2. Transcriptional response to CdS QDs. (a) CdS QD-responsive genes compared to those regulated by Cd^{2+} ions in roots (R) or leaves (L) in *A. thaliana*. QD (I) and QD (II) represent gene expression profiles of plants exposed to two doses of CdS QDs (40 and 80 mg L^{-1}, respectively). (b) Flux through the phenylpropanoid synthesis pathway is enhanced by exposure to CdS QDs but not by exposure to Cd^{2+} ions. Several genes involved in phenylpropanoid synthesis, such as *At5g44630* (encoding terpene synthase and cyclase) and *At4g16740* (ATTPS03, terpene synthase 03), are induced by CdS QDs. (c) Genes associated with photosynthesis are up-regulated by CdS QDs.

Therefore, CuO NPs modulate genes involved in root development and in plant stress response, as well as those implicated in hormone signaling, oxidative stress response and phenylpropanoid biosynthesis. Similar to ZnO NPs, CuO NP treatment affects the expression of genes associated with metal stress, suggesting a release of Cu^{2+} ions from these ENMs.

CADMIUM SULFIDE QUANTUM DOTS

Marmiroli et al.[46,166] characterized the major transcriptomic and proteomic changes associated with exposure to CdS QDs (5 nm diameter) in *Arabidopsis*. CdS QD treatment decreases biomass accumulation, respiration, and chlorophyll content while inducing a reprogramming of the transcription with respect to >1000 genes (63% of which were up-regulated; Figure 2). Various evidence suggests a negligible Cd^{2+} ion release from CdS QDs;[46,167] in fact, neither of the *Arabidopsis* mutants identified as tolerant to CdS QDs shows tolerance to Cd^{2+},[46] and in addition, neither of the Cd^{2+} hypersensitive mutants is hypersensitive to CdS QDs.[168]

Genes encoding antioxidant enzymes are up-regulated in both Cd^{2+} and CdS-QD-treated plants, suggesting that the QDs induce ROS production (Table S1). Plant response to CdS QDs and Cd^{2+} includes the production of anthocyanins, antioxidant pigments able to chelate metals.[54,71,72,169−172] CdS QD exposure represses the genes involved in pectin synthesis (Table S8), and it has been shown that pectin degradation mediated by ROS is promoted by other ENMs.[54] As observed for other ENMs (Tables S3, S4, S6, and S7), CdS QDs down-regulate genes encoding for components of trichoblast differentiation and root development pathways (Table S8).

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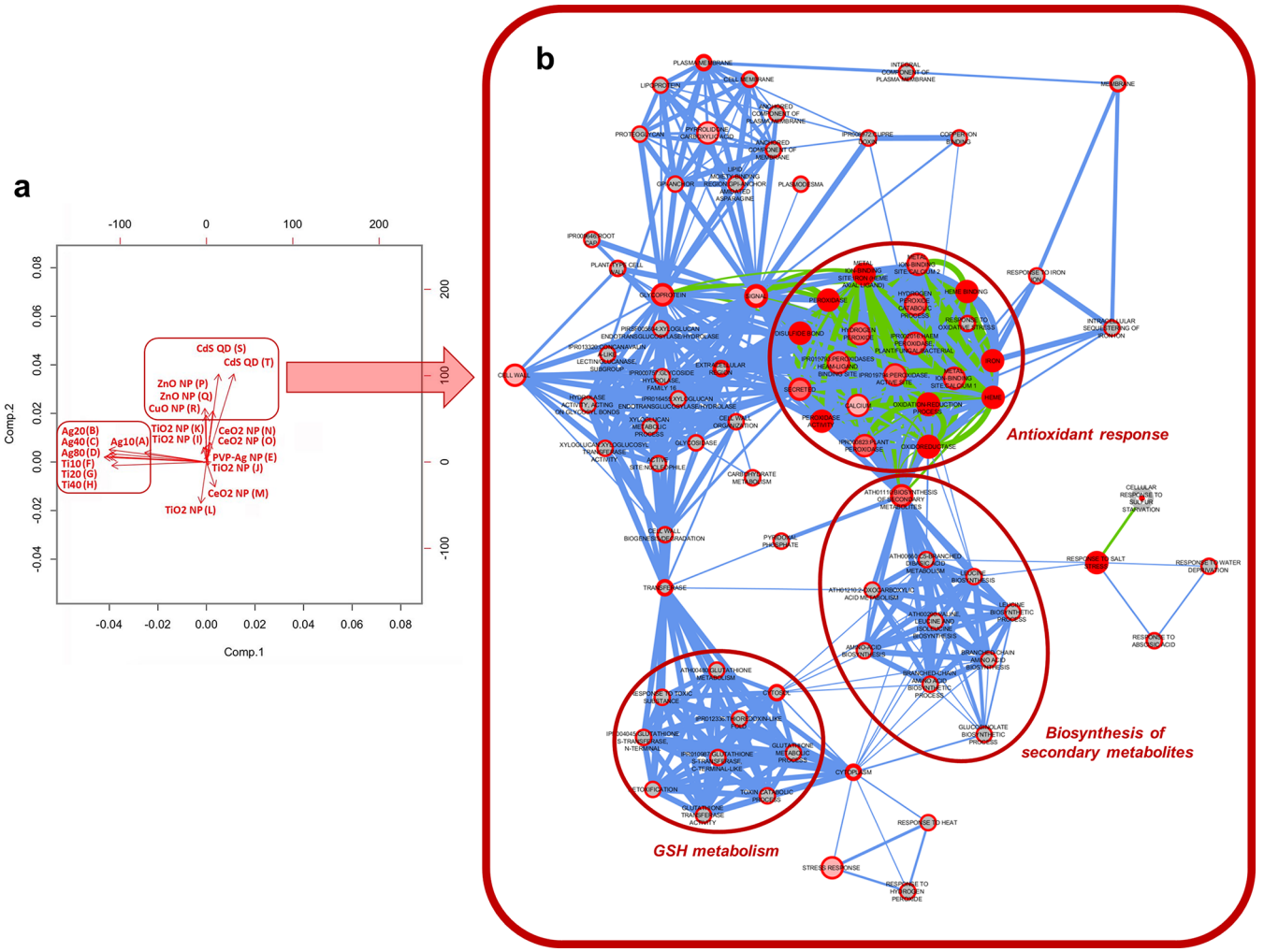

Figure 3. PCA and enrichment analysis and similarities among plant responses to metal-based ENMs. (a) PCA of normalized microarray data considered in this review. Capital letters (A−T) refers to different sets of microarray data enlisted in Table 1: Ag NPs [10 (A), 20 (B), 40 (C), and 80 (D) nm diameter] from García-Sánchez et al.;[70] PVP-Ag NPs (E) from Kaveh et al.;[71] TiO_2 NPs [10 (F), 20 (G), and 40 (H) nm diameter] from García-Sánchez et al;[70] TiO_2 NPs (I) from Landa et al.;[72] TiO_2 NPs (J) from Tumburu et al.;[61] TiO_2 NPs (roots, K; shoots, L) from Tumburu et al.;[73] CeO_2 NPs (M) from Tumburu et al.;[61] CeO_2 NPs (roots, N; shoots, O) from Tumburu et al.;[73] ZnO NPs (P) from Landa et al.;[72] ZnO NPs (Q) from Landa et al.;[74] CuO NPs (R) from Tang et al.[75] and CdS QDs [40 (S) and 80 (T) mg L^{-1}] from Marmiroli et al.[46] PCA plot with relative proportions of variance of overall data set; the first two components (25.6% and 11% of variance, respectively) correspond to data sets from early exposure to Ag and TiO_2 NPs,[70] and data sets from plants treated with ENMs that showed negative effects, as CdS QDs, CuO, and ZnO NPs. (b) Enrichment analysis identify shared response to CdS QDs, CuO, and ZnO NP exposure. Analysis performed with the DAVID Functional Annotation Tool is visualize as a network using the Cytoscape network visualization software. Nodes represent gene sets, and edges represent mutual overlap; highly redundant gene sets are grouped together as clusters. Node (inner circle) size corresponds to the number of genes in data set 1 (modulated genes shared by CdS QDs, CuO NPs, and ZnO NP treatments) within the gene set. Node border (outer circle) size corresponds to the number of genes in data set 2 (shared by two out of three treatments) within the gene set. The color of the node (inner circle) and border (outer circle) correspond to the significance of the gene set for data set 1 and data set 2, respectively. Edge size corresponds to the number of genes that overlap between the two connected gene sets. Green edges correspond to data set 1, and blue corresponds only to data set 2.

The treated plants heightened response to water stress suggests that CdS QD exposure may reduce hydraulic conductivity in the primary root, leading to a reduction in leaf transpiration and growth (Table S8). It is conceivable that physical interactions between CdS QDs and the cell wall lead to increased root lignification, thus compromising root morphology, apoplastic flow and stomatal function.

CdS QD exposure up-regulates genes involved in root sulfate uptake (Table S8). In Cd-treated plants, the increase in sulfate uptake is related to changes in the levels of antioxidant species (e.g., glutathione) and Cd-binding peptides involved in metal detoxification, such as phytochelatins, or is caused by higher turnover of sulfur-containing proteins inactivated by metal stress.[173,174] Possible changes in S-dependent mechanisms may be also implicated in CdS QD stress response. Genes involved in photosynthesis and phenylpropanoid synthesis[107] are up-regulated by the presence of CdS QDs but not by that of the Cd^{2+} ions (Figure 2).

In summary, CdS QDs release negligible amounts of Cd^{2+} ions and the toxicity observed upon CdS QD exposure seems to be associated with the ENM itself. CdS QD treatment decreases biomass, chlorophyll content, and respiratory efficiency in *Arabidopsis* but increases the transcription of gene products involved in antioxidant synthesis, water stress response, photosynthesis, and plant-root development.

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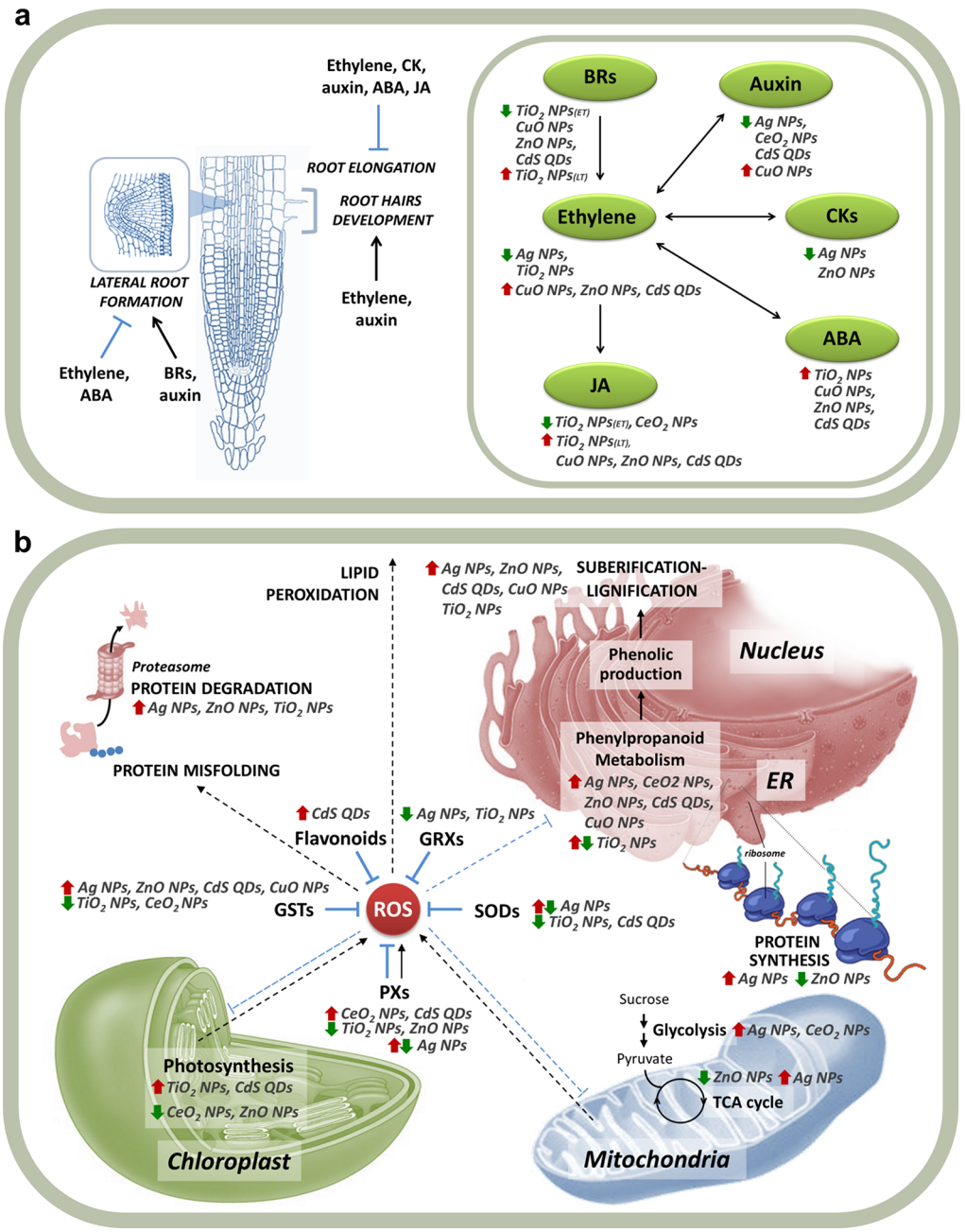

Figure 4. Pathways mediated by metal-based ENMs in plants. (a) Simplified cross-talk diagram showing the interaction between hormone signaling pathways and NP stress response. Hormone-signaling pathways coordinate root growth and lateral root and root-hair development through complex cross-talk. The activities of auxin, ethylene, ABA, JA, cytokinins (CKs), and brassinosteroids (BRs) exhibit either synergistic or antagonistic interactions. TiO_2 NPs (ET), early treatment to nanotitania; TiO_2 NPs (ST), long-term treatment to nanotitania. (b) Schematic illustration of cellular pathways responding to NP stress. PXs, peroxidase proteins; GRXs, glutaredoxin proteins; TCA cycle, tricarboxylic acid cycle. Black arrows indicate stimulatory effects; blue horizontal bars indicate inhibitory effects. Dotted lines represent hypothetical interactions. Up-regulated genes are indicated by red arrows and down-regulated by green arrows.

INTEGRATING "OMICS" APPROACHES FOR METAL-BASED ENM TOXICITY

In this Review, plant response to metal-based ENM exposure has been analyzed comparing data from various "omics" studies[46,61,70−75] to construct a holistic representation of the plant response to ENMs (Figures 3 and 4). Complexity underlying the reported data likely reflects experimental differences regarding the size, dose and aggregation state of the ENMs, as well as the plant developmental stages and time of exposure (Table 1). A further complication is due to the fact that omics studies considered in this review (Table 1) identify very few proteins (Table S2)[78−81] or miRNAs[76,77] modulated by ENM treatments in plants other than *Arabidopsis*, whose molecular functions are often hypothetical.

Response to various metal-based ENMs involves both common and specific pathways, but in general, the toxicity of metal-based ENMs may result from a synergistic action of the metal in nano and ionic forms. In an effort to identify molecular pathways affected by different ENMs and those genes similarly modulated in different conditions, PCA (Figure 3a) was used to assess the relationships between the transcriptomic responses

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induced by ENM treatments. The first and second components of these analysis (Figure 3a), which captured, respectively, 25.6% and 11% of the variation, are represented by the response to early treatments to Ag (10−80 nm) and TiO_2 (10−40 nm) NPs[70] (first component) and ENM treatments that cause negative effects in plant growth (second component), especially CdS QDs (5 nm) but also ZnO (20−100 nm) and CuO (30−50 nm) NPs. Notably, PVP−Ag NP (20 nm) exposure causes negative effects on plant biomass,[71] but the observed transcriptomic changes are not crucial in terms of effects on the total variance of the system (Figure 3a).

Microarray analysis uncovers several genes, e.g., involved in ROS response or root architecture remodeling, whose expression is repressed during the shorter but over-expressed under longer treatments with Ag and TiO_2 NPs. It is possible that early signaling events may influence the capacity of plants to trigger a successful adaptive response and a transcriptional repression of stress-related genes is suspected to be an important molecular mechanism to maintain plant responses under tight control.[175] This hormetic time-response emphasizes a dynamic adaptive response or phenotypic plasticity of the plant following exposure to metal-based ENMs. Among the genes transcriptionally repressed by an early exposure to Ag and TiO_2 NPs, a number are activated by nutrient starvation, water stress and other stimuli that modulate root system architecture (Figure 4 and Tables S3 and S4). Many studies have shown that the exposure to metal-based ENMs affects the nutritional status (e.g., Pi, S, and Fe content) of many crop species.[48,81,105,176−180] S metabolism, normally directed to produce Met and Cys for protein synthesis, can be redirected to the production of GSH, a key element for antioxidant response upon ENM exposure. Nutritional changes associated with ENM exposure are also highlighted by the modulation of several miRNAs (e.g., miR395 and miR399) that regulate these pathways under nanotitania exposure in tobacco plants. Furthermore, a decreased abundance of proteins associated with nutrient storage (e.g., phaseolin) mediated by nanoceria treatment in *P. vulgaris* suggests that ENMs also affect the nutritional quality of seeds.[81]

In addition, PCA plot (Figure 3a) shows that ENM treatments that cause negative effects in *Arabidopsis* plant (CdS QDs, CuO, and ZnO NPs) clustered together. GO analysis of shared genes modulated by these "negative" ENM treatments reveals that several pathways are significantly enriched (Tables S9 and S10 and Figure 3b). "Negative" ENMs cause an overexpression of genes involved in other stress responses (e.g., water deprivation) and in phenylpropanoid metabolism (Figures 3b and 4b), suggesting that an increase in suberification or lignification of plant cell walls can be crucial for the ENM stress response. However, excessive lignification of the cell wall makes mineral and water uptake more difficult, subsequently reducing plant growth and total chlorophyll content.[181]

"Negative" ENMs also increase the expression of genes belonging to hormone signaling pathways[182,183] (Figure 4a and Tables S9 and 10). Ethylene acts primarily to increase cell expansion along the transverse axis,[183] and synergistically with auxin, to promote root-hair formation, inhibiting simultaneous primary root elongation.[184] Lateral root formation is also prevented by ethylene, but it is increased by auxin and brassinosteroids; up-regulation of genes involved in the ethylene signaling pathway is observed in the presence of CdS QDs, CuO, and ZnO NPs (Tables S6−S8 and S10). Notably, an early exposure to Ag and TiO_2 NPs have the opposite effect (Tables S3 and S4). ABA plays a key role in inhibiting lateral root formation when plants are exposed to environmental stress[185] and acts as an antagonist of BR-promoted growth. Genes induced by ABA are up-regulated by CdS QDs and CuO and ZnO NPs, while genes involved in BR biosynthesis are repressed by treatment with these ENMs (Tables S6−S8 and Figure 4a). The major role of JA is in defense against pathogen attack, but this hormone also has a role in plant growth control;[183] the transcription of some JA-responsive genes is increased upon exposure to "negative" ENMs (Tables S6−S8). Genes involved in biosynthesis of SA, a signaling molecule that plays a role in general plant stress response, are down-regulated by an early exposure to Ag and TiO_2 but are up-regulated by exposure to CdS QD and CuO and ZnO NP treatments (Tables S3, S4, S6−S8, and S10).

The "omics" platforms are consistent in predicting that "negative" ENMs induce in *Arabidopsis* an oxidative stress response through ROS production (Tables S9 and S10; Figures 3b and 4b), as reported in crops.[105,162,163] Genes encoding proteins belonging to NADPH oxidase and SODs but mainly peroxidases and GST families, all involved in antioxidant pathways that drive ROS detoxification, are significantly modulated upon CdS QD and CuO and ZnO NP treatments (Figures 3b and 4b). The up-regulation of genes as GSTs can be associated with an elevated S demand in root and leaves.[105] In addition, the biosynthesis of nonenzymatic antioxidants (e.g., flavonoids) is increased upon CdS QD exposure (Tables S8).

Oxidative stress can damage lipids, proteins and DNA and interfere with biochemical reactions, such as reducing photosynthesis.[3,21,39,49−52,54,55] Interestingly, alteration in the transcript or protein levels of key components of protein synthesis and protein degradation are modulated in plants exposed to Ag, TiO_2, and ZnO NPs (Tables S3, S4, and S6; Figure 4b). Conversely, different transcriptional changes are observed in genes involved in synthesis and function of both photosynthetic complexes I and II upon ENM treatments (Figure 4b). Negative and positive effects on chlorophyll synthesis have been observed upon treatment with several ENMs.[3,10,20,46,186−188] A more-practical insight that arises from these molecular studies is related to the possibilities to develop new strategies and predictive tools for assessing exposure and effects (e.g., biomarkers) in plants.[162]

■ ENVIRONMENTAL IMPLICATIONS

Omics approaches have succeeded in identifying certain responses, which point to potential toxicity pathways and to modes of action of ENMs. Exposure to ENMs clearly provokes a generalized stress response, and plenty of evidence favors the notion that oxidative stress is one of its drivers. However, ENM exposure is also associated with the regulation of a suite of genes involved in nutrient uptake and transport, root development, and hormone-signal transduction that induce a range of physiological and morphological changes. This more-holistic view of the plant response may well conflict with outcomes inferred from purely transcriptomic or proteomic data, which impose a largely mechanistic perspective. Here, the approach was to consider omics data derived from a variety of sources, and the issue was how to integrate multiple data sets derived from distinct experimental conditions and based on a number of different ENMs. The comparisons have been further complicated by inherent differences in the size of the omic data sets. This disparity in itself mitigates against any straightforward

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application of multivariate statistics. Nevertheless, some interesting insights have been obtained, as reported in Figures 3 and 4.

Phenotypic data, while being highly informative, suffer from a lack of robustness as reflected in the variability, which arises from the existence of genotype-environment interactions; instead, omics data, while generally robust, tend to be less informative and are essentially descriptive, if not properly deciphered and integrated. It has been frequently noted that transcriptomic and proteomic data are at best only loosely correlated with one another,[166,189] but this discrepancy arises from other processes, notably post-translation modification, which intervene between transcription and protein accumulation. Nevertheless, omics-based analyses do clearly benefit from the use of mutants, just as phenotypic studies do.[46,166] In critically reviewing the existing data, we have not fully considered metabolomic studies, in part because most of these works have been published only recently.[144,145,153,190,191] Indeed, metabolomic profiling analysis is a powerful tool that can provide a deeper insight into the response of complex biological systems under ENM stress. Metabolites in many cases represent the final downstream product of gene expression, and as such, the metabolome is strongly related to the phenotype when a genetic control in the response has to be considered. The incorporation of these omics metadata into networking analysis should promote the integration of mechanistic and holistic views of the living organism.

From an environmental perspective, a thorough understanding of plant response to ENMs is very important. New applications of nanotechnologies in agriculture, which range from crop productivity and nutritional quality to plant protection,[192−195] may in fact pose unpredictable risks associated with the intentional release of ENMs into the environment.[196] This may lead to higher input fluxes than predicted to date.[197]

Therefore, some results discussed suggest caution when advocating the use of metal-based ENMs on crop plants because properties, such as nanoscale size, composition, coating, and application method, may cause problematic effects even when overt toxicity is not evident.[192] In addition, further studies are needed to understand how ENMs are transferred through ecosystems along various pathways, how these materials can cause toxicity to different organisms and communities, including affecting biodiversity, and how transfer within food chains to top level consumers can occur. Many of these questions will need to be addressed prior to the sustainable application of ENMs in agriculture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04121.

Additional details on negative and positive effects of metal-based ENMs in plants is presented. Figures showing a boxplot of the distribution of normalized microarray data, the hierarchical clustering and the functional analysis of TiO_2 NP responsive genes in *A. thaliana*, and the metabolic pathways associated with the transcriptional changes induced by the exposure of *A. thaliana* to ZnO NPs. (PDF)

Tables showing the complete set of gene expression data from plants exposed to metal-based NPs, differentially expressed proteins identified, the GO biological process analysis of ENM modulated genes, and lists of shared genes identified in treatments with negative effects identified by PCA and their GO characterization. (ZIP)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) ASTM. Standard terminology relating to nanotechnology (E2456-06); ASTM International: West Conshohocken, PA, 2006.

(2) Nel, A.; Xia, T.; Madler, L.; Li, N. Toxic potential of materials at the nanolevel. *Science* **2006**, *311* (5761), 622−627.

(3) Ma, C.; White, J. C.; Dhankher, O. P.; Xing, B. Metal-based nanotoxicity and detoxification pathways in higher plants. *Environ. Sci. Technol.* **2015**, *49* (12), 7109−7122.

(4) Maurer-Jones, M. A.; Gunsolus, I. L.; Murphy, C. J.; Haynes, C. L. Toxicity of engineered nanoparticles in the environment. *Anal. Chem.* **2013**, *85* (6), 3036−3049.

(5) Stensberg, M. C.; Wei, Q.; McLamore, E. S.; Porterfield, D. M.; Wei, A.; Sepulveda, M. S. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. *Nanomedicine (London, U. K.)* **2011**, *6* (5), 879−898.

(6) Gottschalk, F.; Nowack, B. The release of engineered nanomaterials to the environment. *J. Environ. Monit.* **2011**, *13* (5), 1145−1155.

(7) Gottschalk, F.; Sun, T.; Nowack, B. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. *Environ. Pollut.* **2013**, *181*, 287−300.

(8) Ma, X.; Geiser-Lee, J.; Deng, Y.; Kolmakov, A. Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Sci. Total Environ.* **2010**, *408* (16), 3053−3061.

(9) Servin, A. D.; White, J. C. Nanotechnology in agriculture: next steps for understanding engineered nanoparticle exposure and risk. *NanoImpact* **2016**, *1*, 9−12.

(10) Dimkpa, C. O.; McLean, J. E.; Latta, D. E.; Manangon, E.; Britt, D. W.; Johnson, W. P.; Boyanov, M. I.; Anderson, A. J. CuO and ZnO nanoparticles: Phytotoxicity, metal speciation and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res.* **2012**, *14*, 1125−1140.

(11) Hong, J.; Peralta-Videa, J. R.; Rico, C.; Sahi, S.; Viveros, M. N.; Bartonjo, J.; Zhao, L.; Gardea-Torresdey, J. L. Evidence of translocation and physiological impacts of foliar applied CeO2 nano-

DOI: 10.1021/acs.est.7b04121
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

particles on cucumber (Cucumis sativus) plants. *Environ. Sci. Technol.* **2014**, *48* (8), 4376−4385.
(12) Larue, C.; Laurette, J.; Herlin-Boime, N.; Khodja, H.; Fayard, B.; Flank, A. M.; Brisset, F.; Carriere, M. Accumulation, translocation and impact of TiO2 nanoparticles in wheat (Triticum aestivum spp.): influence of diameter and crystal phase. *Sci. Total Environ.* **2012**, *431*, 197−208.
(13) Schwab, F.; Zhai, G.; Kern, M.; Turner, A.; Schnoor, J. L.; Wiesner, M. R. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants−Critical review. *Nanotoxicology* **2016**, *10* (3), 257−278.
(14) Zhang, Z.; He, X.; Zhang, H.; Ma, Y.; Zhang, P.; Ding, Y.; Zhao, Y. Uptake and distribution of ceria nanoparticles in cucumber plants. *Metallomics* **2011**, *3* (8), 816−822.
(15) Zhu, Z. J.; Wang, H.; Yan, B.; Zheng, H.; Jiang, Y.; Miranda, O. R.; Rotello, V. M.; Xing, B.; Vachet, R. W. Effect of surface charge on the uptake and distribution of gold nanoparticles in four plant species. *Environ. Sci. Technol.* **2012**, *46* (22), 12391−12398.
(16) Judy, J. D.; Unrine, J. M.; Rao, W.; Wirick, S.; Bertsch, P. M. Bioavailability of gold nanomaterials to plants: importance of particle size and surface coating. *Environ. Sci. Technol.* **2012**, *46* (15), 8467−8474.
(17) Lee, W. M.; An, Y. J.; Yoon, H.; Kweon, H. S. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): plant agar test for water-insoluble nanoparticles. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1915−1921.
(18) Zhu, H.; Han, J.; Xiao, J. Q.; Jin, Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *J. Environ. Monit.* **2008**, *10* (6), 713−717.
(19) Schwabe, F.; Schulin, R.; Limbach, L. K.; Stark, W.; Burge, D.; Nowack, B. Influence of two types of organic matter on interaction of CeO2 nanoparticles with plants in hydroponic culture. *Chemosphere* **2013**, *91* (4), 512−520.
(20) Servin, A. D.; Morales, M. I.; Castillo-Michel, H.; Hernández-Viezcas, J. A.; Munoz, B.; Zhao, L.; Nunez, J. E.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Synchrotron verification of TiO2 accumulation in cucumber fruit: a possible pathway of TiO2 nanoparticle transfer from soil into the food chain. *Environ. Sci. Technol.* **2013**, *47* (20), 11592−11598.
(21) Atha, D. H.; Wang, H.; Petersen, E. J.; Cleveland, D.; Holbrook, R. D.; Jaruga, P.; Dizdaroglu, M.; Xing, B.; Nelson, B. C. Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environ. Sci. Technol.* **2012**, *46* (3), 1819−1827.
(22) Wang, Q.; Ma, X.; Zhang, W.; Pei, H.; Chen, Y. The impact of cerium oxide nanoparticles on tomato (Solanum lycopersicum L.) and its implications for food safety. *Metallomics* **2012**, *4* (10), 1105−1112.
(23) Nhan, L. V.; Ma, C.; Rui, Y.; Liu, S.; Li, X.; Xing, B.; Liu, L. Phytotoxic Mechanism of Nanoparticles: Destruction of Chloroplasts and Vascular Bundles and Alteration of Nutrient Absorption. *Sci. Rep.* **2015**, *5*, 11618.
(24) Lin, D.; Xing, B. Root uptake and phytotoxicity of ZnO nanoparticles. *Environ. Sci. Technol.* **2008**, *42* (15), 5580−5585.
(25) Zhang, D.; Hua, T.; Xiao, F.; Chen, C.; Gersberg, R. M.; Liu, Y.; Stuckey, D.; Ng, W. J.; Tan, S. K. Phytotoxicity and bioaccumulation of ZnO nanoparticles in Schoenoplectus tabernaemontani. *Chemosphere* **2015**, *120*, 211−219.
(26) Lin, D.; Xing, B. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ. Pollut.* **2007**, *150* (2), 243−250.
(27) Lee, C. W.; Mahendra, S.; Zodrow, K.; Li, D.; Tsai, Y. C.; Braam, J.; Alvarez, P. J. Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. *Environ. Toxicol. Chem.* **2010**, *29* (3), 669−675.
(28) Josko, I.; Oleszczuk, P. Phytotoxicity of nanoparticles−problems with bioassay choosing and sample preparation. *Environ. Sci. Pollut. Res.* **2014**, *21* (17), 10215−10224.
(29) Handy, R. D.; Owen, R.; Valsami-Jones, E. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* **2008**, *17* (5), 315−325.
(30) Lee, W. M.; Kwak, J. I.; An, Y. J. Effect of silver nanoparticles in crop plants Phaseolus radiatus and Sorghum bicolor: media effect on phytotoxicity. *Chemosphere* **2012**, *86* (5), 491−499.
(31) Majumdar, S.; Peralta-Videa, J. R.; Trujillo-Reyes, J.; Sun, Y.; Barrios, A. C.; Niu, G.; Margez, J. P.; Gardea-Torresdey, J. L. Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide nanoparticles. *Sci. Total Environ.* **2016**, *569−570*, 201−211.
(32) Zhao, L.; Peralta-Videa, J. R.; Varela-Ramirez, A.; Castillo-Michel, H.; Li, C.; Zhang, J.; Aguilera, R. J.; Keller, A. A.; Gardea-Torresdey, J. L. Effect of surface coating and organic matter on the uptake of CeO2 NPs by corn plants grown in soil: Insight into the uptake mechanism. *J. Hazard. Mater.* **2012**, *225−226*, 131−138.
(33) Feng, Y.; Cui, X.; He, S.; Dong, G.; Chen, M.; Wang, J.; Lin, X. The role of metal nanoparticles in influencing arbuscular mycorrhizal fungi effects on plant growth. *Environ. Sci. Technol.* **2013**, *47* (16), 9496−9504.
(34) Yang, L.; Watts, D. J. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol. Lett.* **2005**, *158* (2), 122−132.
(35) Stampoulis, D.; Sinha, S. K.; White, J. C. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* **2009**, *43* (24), 9473−9479.
(36) López-Moreno, M. L.; de la Rosa, G.; Hernández-Viezcas, J. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO(2) nanoparticles and assessment of their differential toxicity in four edible plant species. *J. Agric. Food Chem.* **2010**, *58* (6), 3689−3693.
(37) Ma, Y.; Kuang, L.; He, X.; Bai, W.; Ding, Y.; Zhang, Z.; Zhao, Y.; Chai, Z. Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere* **2010**, *78* (3), 273−279.
(38) Du, W.; Sun, Y.; Ji, R.; Zhu, J.; Wu, J.; Guo, H. TiO2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. *J. Environ. Monit.* **2011**, *13* (4), 822−828.
(39) Kumari, M.; Khan, S. S.; Pakrashi, S.; Mukherjee, A.; Chandrasekaran, N. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa. *J. Hazard. Mater.* **2011**, *190* (1−3), 613−621.
(40) El-Temsah, Y. S.; Joner, E. J. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. *Environ. Toxicol.* **2012**, *27* (1), 42−49.
(41) Geisler-Lee, J.; Wang, Q.; Yao, Y.; Zhang, W.; Geisler, M.; Li, K.; Huang, Y.; Chen, Y.; Kolmakov, A.; Ma, X. Phytotoxicity, accumulation and transport of silver nanoparticles by Arabidopsis thaliana. *Nanotoxicology* **2012**, *7* (3), 323−337.
(42) Pokhrel, L. R.; Dubey, B. Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles. *Sci. Total Environ.* **2013**, *452−453*, 321−332.
(43) Nair, P. M.; Chung, I. M. Assessment of silver nanoparticle-induced physiological and molecular changes in Arabidopsis thaliana. *Environ. Sci. Pollut. Res.* **2014**, *21* (14), 8858−8869.
(44) Zuverza-Mena, N.; Martinez-Fernandez, D.; Du, W.; Hernandez-Viezcas, J. A.; Bonilla-Bird, N.; Lopez-Moreno, M. L.; Komarek, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. *Plant Physiol. Biochem.* **2017**, *110*, 236−264.
(45) Rico, C. M.; Majumdar, S.; Duarte-Gardea, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food Chem.* **2011**, *59* (8), 3485−3498.
(46) Marmiroli, M.; Pagano, L.; Savo Sardaro, M. L.; Villani, M.; Marmiroli, N. Genome-wide approach in Arabidopsis thaliana to assess the toxicity of cadmium sulfide quantum dots. *Environ. Sci. Technol.* **2014**, *48* (10), 5902−5909.

DOI: 10.1021/acs.est.7b04121
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(47) Asli, S.; Neumann, P. M. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. *Plant, Cell Environ.* **2009**, *32* (5), 577−584.
(48) Martinez-Fernandez, D.; Barroso, D.; Komarek, M. Root water transport of Helianthus annuus L. under iron oxide nanoparticle exposure. *Environ. Sci. Pollut. Res.* **2016**, *23* (2), 1732−1741.
(49) Kumari, M.; Mukherjee, A.; Chandrasekaran, N. Genotoxicity of silver nanoparticles in Allium cepa. *Sci. Total Environ.* **2009**, *407* (19), 5243−5246.
(50) Ghosh, M.; Bandyopadhyay, M.; Mukherjee, A. Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophic levels: plant and human lymphocytes. *Chemosphere* **2010**, *81* (10), 1253−1262.
(51) Pakrashi, S.; Jain, N.; Dalai, S.; Jayakumar, J.; Chandrasekaran, P. T.; Raichur, A. M.; Chandrasekaran, N.; Mukherjee, A. In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. *PLoS One* **2014**, *9* (2), e87789.
(52) Thiruvengadam, M.; Gurunathan, S.; Chung, I. M. Physiological, metabolic, and transcriptional effects of biologically-synthesized silver nanoparticles in turnip (Brassica rapa ssp. rapa L.). *Protoplasma* **2015**, *252* (4), 1031−1046.
(53) Khan, M. N.; Mobin, M.; Abbas, Z. K.; AlMutairi, K. A.; Siddiqui, Z. H. Role of nanomaterials in plants under challenging environments. *Plant Physiol. Biochem.* **2017**, *110*, 194−209.
(54) Kim, J. H.; Lee, Y.; Kim, E. J.; Gu, S.; Sohn, E. J.; Seo, Y. S.; An, H. J.; Chang, Y. S. Exposure of iron nanoparticles to Arabidopsis thaliana enhances root elongation by triggering cell wall loosening. *Environ. Sci. Technol.* **2014**, *48* (6), 3477−3485.
(55) Ghosh, M.; J, M.; Sinha, S.; Chakraborty, A.; Mallick, S. K.; Bandyopadhyay, M.; Mukherjee, A. In vitro and in vivo genotoxicity of silver nanoparticles. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2012**, *749* (1−2), 60−69.
(56) Kumar, V.; Guleria, P.; Yadav, S. K.; Kumar, V. Gold nanoparticle exposure induces growth and yield enhancement in Arabidopsis thaliana. *Sci. Total Environ.* **2013**, *461*−*462*, 462−468.
(57) Hong, F.; Zhou, J.; Liu, C.; Yang, F.; Wu, C.; Zheng, L.; Yang, P. Effect of nano-TiO2 on photochemical reaction of chloroplasts of spinach. *Biol. Trace Elem. Res.* **2005**, *105* (1−3), 269−279.
(58) Su, M.; Liu, H.; Liu, C.; Qu, C.; Zheng, L.; Hong, F. Promotion of nano-anatase TiO(2) on the spectral responses and photochemical activities of D1/D2/Cyt b559 complex of spinach. *Spectrochim. Acta, Part A* **2009**, *72* (5), 1112−1116.
(59) Sharma, P.; Bhatt, D.; Zaidi, M. G.; Saradhi, P. P.; Khanna, P. K.; Arora, S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of Brassica juncea. *Appl. Biochem. Biotechnol.* **2012**, *167* (8), 2225−2233.
(60) Yang, F.; Hong, F.; You, W.; Liu, C.; Gao, F.; Wu, C.; Yang, P. Influences of nano-anatase TiO2 on the nitrogen metabolism of growing spinach. *Biol. Trace Elem. Res.* **2006**, *110* (2), 179−190.
(61) Tumburu, L.; Andersen, C. P.; Rygiewicz, P. T.; Reichman, J. R. Phenotypic and genomic responses to titanium dioxide and cerium oxide nanoparticles in Arabidopsis germinants. *Environ. Toxicol. Chem.* **2015**, *34* (1), 70−83.
(62) Wang, Q.; Ebbs, S. D.; Chen, Y.; Ma, X. Trans-generational impact of cerium oxide nanoparticles on tomato plants. *Metallomics* **2013**, *5* (6), 753−759.
(63) Gardea-Torresdey, J. L.; Rico, C. M.; White, J. C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* **2014**, *48* (5), 2526−2540.
(64) Cox, A.; Venkatachalam, P.; Sahi, S.; Sharma, N. Silver and titanium dioxide nanoparticle toxicity in plants: A review of current research. *Plant Physiol. Biochem.* **2016**, *107*, 147−163.
(65) de la Rosa, G.; Garcia-Castaneda, C.; Vazquez-Nunez, E.; Alonso-Castro, A. J.; Basurto-Islas, G.; Mendoza, A.; Cruz-Jimenez, G.; Molina, C. Physiological and biochemical response of plants to engineered NMs: Implications on future design. *Plant Physiol. Biochem.* **2017**, *110*, 226−235.
(66) Rizwan, M.; Ali, S.; Qayyum, M. F.; Ok, Y. S.; Adrees, M.; Ibrahim, M.; Zia-Ur-Rehman, M.; Farid, M.; Abbas, F. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. *J. Hazard. Mater.* **2017**, *322*, 2−16.
(67) Mustafa, G.; Komatsu, S. Toxicity of heavy metals and metal-containing nanoparticles on plants. *Biochim. Biophys. Acta, Proteins Proteomics* **2016**, *1864* (8), 932−944.
(68) Siddiqi, K. S.; Husen, A. Plant Response to Engineered Metal Oxide Nanoparticles. *Nanoscale Res. Lett.* **2017**, *12* (1), 92.
(69) Du, W.; Tan, W.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L.; Ji, R.; Yin, Y.; Guo, H. Interaction of metal oxide nanoparticles with higher terrestrial plants: Physiological and biochemical aspects. *Plant Physiol. Biochem.* **2017**, *110*, 210−225.
(70) García-Sánchez, S.; Bernales, I.; Cristobal, S. Early response to nanoparticles in the Arabidopsis transcriptome compromises plant defence and root-hair development through salicylic acid signalling. *BMC Genomics* **2015**, *16*, 341.
(71) Kaveh, R.; Li, Y. S.; Ranjbar, S.; Tehrani, R.; Brueck, C. L.; Van Aken, B. Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions. *Environ. Sci. Technol.* **2013**, *47* (18), 10637−10644.
(72) Landa, P.; Vankova, R.; Andrlova, J.; Hodek, J.; Marsik, P.; Storchova, H.; White, J. C.; Vanek, T. Nanoparticle-specific changes in Arabidopsis thaliana gene expression after exposure to ZnO, TiO2, and fullerene soot. *J. Hazard. Mater.* **2012**, *241*−*242*, 55−62.
(73) Tumburu, L.; Andersen, C. P.; Rygiewicz, P. T.; Reichman, J. R. Molecular and physiological responses to titanium dioxide and cerium oxide nanoparticles in Arabidopsis. *Environ. Toxicol. Chem.* **2017**, *36* (1), 71−82.
(74) Landa, P.; Prerostova, S.; Petrova, S.; Knirsch, V.; Vankova, R.; Vanek, T. The Transcriptomic Response of Arabidopsis thaliana to Zinc Oxide: A Comparison of the Impact of Nanoparticle, Bulk, and Ionic Zinc. *Environ. Sci. Technol.* **2015**, *49* (24), 14537−14545.
(75) Tang, Y.; He, R.; Zhao, J.; Nie, G.; Xu, L.; Xing, B. Oxidative stress-induced toxicity of CuO nanoparticles and related toxicogenomic responses in Arabidopsis thaliana. *Environ. Pollut.* **2016**, *212*, 605−614.
(76) Frazier, T. P.; Burklew, C. E.; Zhang, B. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (Nicotiana tabacum). *Funct. Integr. Genomics* **2014**, *14* (1), 75−83.
(77) Burklew, C. E.; Ashlock, J.; Winfrey, W. B.; Zhang, B. Effects of aluminum oxide nanoparticles on the growth, development, and microRNA expression of tobacco (Nicotiana tabacum). *PLoS One* **2012**, *7* (5), e34783.
(78) Vannini, C.; Domingo, G.; Onelli, E.; Prinsi, B.; Marsoni, M.; Espen, L.; Bracale, M. Morphological and proteomic responses of Eruca sativa exposed to silver nanoparticles or silver nitrate. *PLoS One* **2013**, *8* (7), e68752.
(79) Mirzajani, F.; Askari, H.; Hamzelou, S.; Schober, Y.; Rompp, A.; Ghassempour, A.; Spengler, B. Proteomics study of silver nanoparticles toxicity on Oryza sativa L. *Ecotoxicol. Environ. Saf.* **2014**, *108*, 335−339.
(80) Vannini, C.; Domingo, G.; Onelli, E.; De Mattia, F.; Bruni, I.; Marsoni, M.; Bracale, M. Phytotoxic and genotoxic effects of silver nanoparticles exposure on germinating wheat seedlings. *J. Plant Physiol.* **2014**, *171* (13), 1142−1148.
(81) Majumdar, S.; Almeida, I. C.; Arigi, E. A.; Choi, H.; VerBerkmoes, N. C.; Trujillo-Reyes, J.; Flores-Margez, J. P.; White, J. C.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Environmental Effects of Nanoceria on Seed Production of Common Bean (Phaseolus vulgaris): A Proteomic Analysis. *Environ. Sci. Technol.* **2015**, *49* (22), 13283−13293.
(82) Van Hummelen, P.; Sasaki, J. State-of-the-art genomics approaches in toxicology. *Mutat. Res., Rev. Mutat. Res.* **2010**, *705* (3), 165−171.
(83) Klaper, R.; Arndt, D.; Bozich, J.; Dominguez, G. Molecular interactions of nanomaterials and organisms: defining biomarkers for

DOI: 10.1021/acs.est.7b04121
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

toxicity and high-throughput screening using traditional and next-generation sequencing approaches. *Analyst* **2014**, *139* (5), 882−895.
(84) Scott-Fordsmand, J. J.; Peijnenburg, W.; Amorim, M. J.; Landsiedel, R.; Oorts, K. The way forward for risk assessment of nanomaterials in solid media. *Environ. Pollut.* **2016**, *218*, 1363−1364.
(85) Hjorth, R.; Holden, P. A.; Hansen, S. F.; Colman, B. P.; Grieger, K.; Hendren, C. O. The role of alternative testing strategies in environmental risk assessment of engineered nanomaterials. *Environ. Sci.: Nano* **2017**, *4*, 292−301.
(86) Eisen, M. B.; Spellman, P. T.; Brown, P. O.; Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95* (25), 14863−14868.
(87) Saldanha, A. J. Java Treeview−extensible visualization of microarray data. *Bioinformatics* **2004**, *20* (17), 3246−3248.
(88) Thimm, O.; Blasing, O.; Gibon, Y.; Nagel, A.; Meyer, S.; Kruger, P.; Selbig, J.; Muller, L. A.; Rhee, S. Y.; Stitt, M. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* **2004**, *37* (6), 914−939.
(89) Merico, D.; Isserlin, R.; Stueker, O.; Emili, A.; Bader, G. D. Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PLoS One* **2010**, *5* (11), e13984.
(90) Dennis, G., Jr.; Sherman, B. T.; Hosack, D. A.; Yang, J.; Gao, W.; Lane, H. C.; Lempicki, R. A. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* **2003**, *4* (5), P3.
(91) Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N. S.; Wang, J. T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **2003**, *13* (11), 2498−2504.
(92) Shen, X. S.; Wang, G. Z.; Hong, X.; Zhu, W. Nanospheres of silver nanoparticles: agglomeration, surface morphology control and application as SERS substrates. *Phys. Chem. Chem. Phys.* **2009**, *11* (34), 7450−7454.
(93) Yin, L.; Cheng, Y.; Espinasse, B.; Colman, B. P.; Auffan, M.; Wiesner, M.; Rose, J.; Liu, J.; Bernhardt, E. S. More than the ions: the effects of silver nanoparticles on Lolium multiflorum. *Environ. Sci. Technol.* **2011**, *45* (6), 2360−2367.
(94) Geisler-Lee, J.; Brooks, M.; Gerfen, J. R.; Wang, Q.; Fotis, C.; Sparer, A.; Ma, X.; Berg, R. H.; Geisler, M. Reproductive toxicity and life history study of silver nanoparticle effect, uptake and transport in Arabidopsis thaliana. *Nanomaterials* **2014**, *4*, 301−318.
(95) Syu, Y. Y.; Hung, J. H.; Chen, J. C.; Chuang, H. W. Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression. *Plant Physiol. Biochem.* **2014**, *83*, 57−64.
(96) Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **2004**, *9* (10), 490−498.
(97) Nair, P. M.; Chung, I. M. Physiological and molecular level effects of silver nanoparticles exposure in rice (Oryza sativa L.) seedlings. *Chemosphere* **2014**, *112*, 105−113.
(98) Tanimoto, M.; Roberts, K.; Dolan, L. Ethylene is a positive regulator of root hair development in Arabidopsis thaliana. *Plant J.* **1995**, *8* (6), 943−948.
(99) Tanaka, N.; Kato, M.; Tomioka, R.; Kurata, R.; Fukao, Y.; Aoyama, T.; Maeshima, M. Characteristics of a root hair-less line of Arabidopsis thaliana under physiological stresses. *J. Exp. Bot.* **2014**, *65* (6), 1497−1512.
(100) Péret, B.; Clément, M.; Nussaume, L.; Desnos, T. Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends Plant Sci.* **2011**, *16* (8), 442−450.
(101) Cruz-Ramirez, A.; Oropeza-Aburto, A.; Razo-Hernandez, F.; Ramirez-Chavez, E.; Herrera-Estrella, L. Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (17), 6765−6770.
(102) Benning, C.; Ohta, H. Three enzyme systems for galactoglycerolipid biosynthesis are coordinately regulated in plants. *J. Biol. Chem.* **2005**, *280* (4), 2397−2400.
(103) Moons, A. Regulatory and functional interactions of plant growth regulators and plant glutathione S-transferases (GSTs). *Vitam. Horm.* **2005**, *72*, 155−202.
(104) Nair, P. M.; Park, S. Y.; Choi, J. Evaluation of the effect of silver nanoparticles and silver ions using stress responsive gene expression in Chironomus riparius. *Chemosphere* **2013**, *92* (5), 592−599.
(105) Tiwari, M.; Sharma, N. C.; Fleischmann, P.; Burbage, J.; Venkatachalam, P.; Sahi, S. V. Nanotitania Exposure Causes Alterations in Physiological, Nutritional and Stress Responses in Tomato (Solanum lycopersicum). *Front. Plant Sci.* **2017**, *8*, 633.
(106) Field, B.; Osbourn, A. E. Metabolic diversification−independent assembly of operon-like gene clusters in different plants. *Science* **2008**, *320* (5875), 543−547.
(107) Dixon, R. A.; Paiva, N. L. Stress-Induced Phenylpropanoid Metabolism. *Plant Cell* **1995**, *7* (7), 1085−1097.
(108) Vogt, T. Phenylpropanoid biosynthesis. *Mol. Plant* **2010**, *3* (1), 2−20.
(109) Vishwanath, S. J.; Delude, C.; Domergue, F.; Rowland, O. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. *Plant Cell Rep.* **2015**, *34* (4), 573−586.
(110) Le Gall, H.; Philippe, F.; Domon, J. M.; Gillet, F.; Pelloux, J.; Rayon, C. Cell Wall Metabolism in Response to Abiotic Stress. *Plants* **2015**, *4* (4), 112−166.
(111) Boerjan, W.; Ralph, J.; Baucher, M. Lignin biosynthesis. *Annu. Rev. Plant Biol.* **2003**, *54*, 519−546.
(112) Wang, Y.; Chantreau, M.; Sibout, R.; Hawkins, S. Plant cell wall lignification and monolignol metabolism. *Front. Plant Sci.* **2013**, *4*, 220.
(113) Kissen, R.; Bones, A. M. Nitrile-specifier proteins involved in glucosinolate hydrolysis in Arabidopsis thaliana. *J. Biol. Chem.* **2009**, *284* (18), 12057−12070.
(114) Falk, K. L.; Tokuhisa, J. G.; Gershenzon, J. The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. *Plant Biol. (Berlin, Ger.)* **2007**, *9* (5), 573−581.
(115) Gondikas, A. P.; Morris, A.; Reinsch, B. C.; Marinakos, S. M.; Lowry, G. V.; Hsu-Kim, H. Cysteine-induced modifications of zero-valent silver nanomaterials: implications for particle surface chemistry, aggregation, dissolution, and silver speciation. *Environ. Sci. Technol.* **2012**, *46* (13), 7037−7045.
(116) Bertea, C. M.; Narayana, R.; Agliassa, C.; Rodgers, C. T.; Maffei, M. E. Geomagnetic Field (Gmf) and Plant Evolution: Investigating the Effects of Gmf Reversal on Arabidopsis thaliana Development and Gene Expression. *J. Visualized Exp.* **2015**, DOI: 10.3791/53286.
(117) Xu, Z.; Escamilla-Trevino, L.; Zeng, L.; Lalgondar, M.; Bevan, D.; Winkel, B.; Mohamed, A.; Cheng, C. L.; Shih, M. C.; Poulton, J.; Esen, A. Functional genomic analysis of Arabidopsis thaliana glycoside hydrolase family 1. *Plant Mol. Biol.* **2004**, *55* (3), 343−367.
(118) Knight, H. Calcium signaling during abiotic stress in plants. *Int. Rev. Cytol.* **1999**, *195*, 269−324.
(119) Yang, T.; Poovaiah, B. W. Calcium/calmodulin-mediated signal network in plants. *Trends Plant Sci.* **2003**, *8* (10), 505−512.
(120) Tesfaye, M.; Temple, S. J.; Allan, D. L.; Vance, C. P.; Samac, D. A. Overexpression of malate dehydrogenase in transgenic alfalfa enhances organic acid synthesis and confers tolerance to aluminum. *Plant Physiol.* **2001**, *127* (4), 1836−1844.
(121) Zhao, L.; Huang, Y.; Hu, J.; Zhou, H.; Adeleye, A. S.; Keller, A. A. (1)H NMR and GC-MS Based Metabolomics Reveal Defense and Detoxification Mechanism of Cucumber Plant under Nano-Cu Stress. *Environ. Sci. Technol.* **2016**, *50* (4), 2000−2010.
(122) Chen, Q.; Guo, C. L.; Wang, P.; Chen, X. Q.; Wu, K. H.; Li, K. Z.; Yu, Y. X.; Chen, L. M. Up-regulation and interaction of the plasma membrane H(+)-ATPase and the 14−3-3 protein are involved in the regulation of citrate exudation from the broad bean (Vicia faba L.) under Al stress. *Plant Physiol. Biochem.* **2013**, *70*, 504−511.
(123) Rouhier, N.; Lemaire, S. D.; Jacquot, J. P. The role of glutathione in photosynthetic organisms: emerging functions for

DOI: 10.1021/acs.est.7b04121
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

glutaredoxins and glutathionylation. *Annu. Rev. Plant Biol.* **2008**, *59*, 143−166.
(124) Harada, E.; Kim, J. A.; Meyer, A. J.; Hell, R.; Clemens, S.; Choi, Y. E. Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant Cell Physiol.* **2010**, *51* (10), 1627−1637.
(125) Osakabe, Y.; Osakabe, K.; Shinozaki, K.; Tran, L. S. Response of plants to water stress. *Front. Plant Sci.* **2014**, *5*, 86.
(126) Servin, A. D.; Castillo-Michel, H.; Hernández-Viezcas, J. A.; Diaz, B. C.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of TiO(2) nanoparticles in cucumber (Cucumis sativus) plants. *Environ. Sci. Technol.* **2012**, *46* (14), 7637−7643.
(127) Mourato, M. P.; Moreira, I. N.; Leitao, I.; Pinto, F. R.; Sales, J. R.; Martins, L. L. Effect of Heavy Metals in Plants of the Genus Brassica. *Int. J. Mol. Sci.* **2015**, *16* (8), 17975−17998.
(128) Zheng, L.; Hong, F.; Lu, S.; Liu, C. Effect of nano-TiO(2) on strength of naturally aged seeds and growth of spinach. *Biol. Trace Elem. Res.* **2005**, *104* (1), 83−92.
(129) Gao, F.; Hong, F.; Liu, C.; Zheng, L.; Su, M.; Wu, X.; Yang, F.; Wu, C.; Yang, P. Mechanism of nano-anatase TiO2 on promoting photosynthetic carbon reaction of spinach: inducing complex of rubisco-rubisco activase. *Biol. Trace Elem. Res.* **2006**, *111* (1−3), 239−253.
(130) Wang, S.; Kurepa, J.; Smalle, J. A. Ultra-small TiO(2) nanoparticles disrupt microtubular networks in Arabidopsis thaliana. *Plant, Cell Environ.* **2011**, *34* (5), 811−820.
(131) Gheshlaghi, Z. N.; Riazi, G. H.; Ahmadian, S.; Ghafari, M.; Mahinpour, R. Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein. *Acta Biochim. Biophys. Sin.* **2008**, *40* (9), 777−782.
(132) Jones-Rhoades, M. W.; Bartel, D. P.; Bartel, B. MicroRNAS and their regulatory roles in plants. *Annu. Rev. Plant Biol.* **2006**, *57*, 19−53.
(133) Liang, G.; Ai, Q.; Yu, D. Uncovering miRNAs involved in crosstalk between nutrient deficiencies in Arabidopsis. *Sci. Rep.* **2015**, *5*, 11813.
(134) Chiou, T. J. The role of microRNAs in sensing nutrient stress. *Plant, Cell Environ.* **2007**, *30* (3), 323−332.
(135) Jones-Rhoades, M. W.; Bartel, D. P. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Mol. Cell* **2004**, *14* (6), 787−799.
(136) Hsieh, L. C.; Lin, S. I.; Shih, A. C.; Chen, J. W.; Lin, W. Y.; Tseng, C. Y.; Li, W. H.; Chiou, T. J. Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. *Plant Physiol.* **2009**, *151* (4), 2120−2132.
(137) Lin, S. I.; Chiou, T. J. Long-distance movement and differential targeting of microRNA399s. *Plant Signaling Behav.* **2008**, *3* (9), 730−732.
(138) Li, W. X.; Oono, Y.; Zhu, J.; He, X. J.; Wu, J. M.; Iida, K.; Lu, X. Y.; Cui, X.; Jin, H.; Zhu, J. K. The Arabidopsis NFYA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance. *Plant Cell* **2008**, *20* (8), 2238−2251.
(139) Zhang, X.; Zou, Z.; Gong, P.; Zhang, J.; Ziaf, K.; Li, H.; Xiao, F.; Ye, Z. Over-expression of microRNA169 confers enhanced drought tolerance to tomato. *Biotechnol. Lett.* **2011**, *33* (2), 403−409.
(140) Reyes, J. L.; Chua, N. H. ABA induction of miR159 controls transcript levels of two MYB factors during Arabidopsis seed germination. *Plant J.* **2007**, *49* (4), 592−606.
(141) Ng, D. W.; Zhang, C.; Miller, M.; Palmer, G.; Whiteley, M.; Tholl, D.; Chen, Z. J. cis- and trans-Regulation of miR163 and target genes confers natural variation of secondary metabolites in two Arabidopsis species and their allopolyploids. *Plant Cell* **2011**, *23* (5), 1729−1740.
(142) Weigel, M.; Varotto, C.; Pesaresi, P.; Finazzi, G.; Rappaport, F.; Salamini, F.; Leister, D. Plastocyanin is indispensable for photosynthetic electron flow in Arabidopsis thaliana. *J. Biol. Chem.* **2003**, *278* (33), 31286−31289.
(143) Ranocha, P.; Chabannes, M.; Chamayou, S.; Danoun, S.; Jauneau, A.; Boudet, A. M.; Goffner, D. Laccase down-regulation causes alterations in phenolic metabolism and cell wall structure in poplar. *Plant Physiol.* **2002**, *129* (1), 145−155.
(144) Wu, B.; Zhu, L.; Le, X. C. Metabolomics analysis of TiO2 nanoparticles induced toxicological effects on rice (Oryza sativa L.). *Environ. Pollut.* **2017**, *230*, 302−310.
(145) Zahra, Z.; Waseem, N.; Zahra, R.; Lee, H.; Badshah, M. A.; Mehmood, A.; Choi, H. K.; Arshad, M. Growth and Metabolic Responses of Rice (Oryza sativa L.) Cultivated in Phosphorus-Deficient Soil Amended with TiO2 Nanoparticles. *J. Agric. Food Chem.* **2017**, *65* (28), 5598−5606.
(146) Bhaskara, G. B.; Yang, T. H.; Verslues, P. E. Dynamic proline metabolism: importance and regulation in water limited environments. *Front. Plant Sci.* **2015**, *6*, 484.
(147) Labavitch, J. M.; Ray, P. M. Relationship between Promotion of Xyloglucan Metabolism and Induction of Elongation by Indole-acetic Acid. *Plant Physiol.* **1974**, *54* (4), 499−502.
(148) Soga, K.; Wakabayashi, K.; Kamisaka, S.; Hoson, T. Stimulation of elongation growth and xyloglucan breakdown in Arabidopsis hypocotyls under microgravity conditions in space. *Planta* **2002**, *215* (6), 1040−1046.
(149) Alboresi, A.; Gestin, C.; Leydecker, M. T.; Bedu, M.; Meyer, C.; Truong, H. N. Nitrate, a signal relieving seed dormancy in Arabidopsis. *Plant, Cell Environ.* **2005**, *28* (4), 500−512.
(150) Schopfer, P.; Plachy, C.; Frahry, G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol.* **2001**, *125* (4), 1591−1602.
(151) Peumans, W. J.; Van Damme, E. J. Lectins as plant defense proteins. *Plant Physiol.* **1995**, *109* (2), 347−352.
(152) Schenk, G.; Mitic, N.; Hanson, G. R.; Comba, P. Purple acid phosphatase: a journey into the function and mechanism of a colorful enzyme. *Coord. Chem. Rev.* **2013**, *257* (2), 473−482.
(153) Salehi, H.; Chehregani, A.; Lucini, L.; Majd, A.; Gholami, M. Morphological, proteomic and metabolomic insight into the effect of cerium dioxide nanoparticles to Phaseolus vulgaris L. under soil or foliar application. *Sci. Total Environ.* **2018**, *616−617*, 1540−1551.
(154) Becher, M.; Talke, I. N.; Krall, L.; Kramer, U. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator Arabidopsis halleri. *Plant J.* **2004**, *37* (2), 251−268.
(155) Li, M.; Ahammed, G. J.; Li, C.; Bao, X.; Yu, J.; Huang, C.; Yin, H.; Zhou, J. Brassinosteroid Ameliorates Zinc Oxide Nanoparticles-Induced Oxidative Stress by Improving Antioxidant Potential and Redox Homeostasis in Tomato Seedling. *Front. Plant Sci.* **2016**, *7*, 615.
(156) Rubio, V.; Linhares, F.; Solano, R.; Martin, A. C.; Iglesias, J.; Leyva, A.; Paz-Ares, J. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev.* **2001**, *15* (16), 2122−2133.
(157) Marmiroli, M.; Antonioli, G.; Maestri, E.; Marmiroli, N. Evidence of the involvement of plant ligno-cellulosic structure in the sequestration of Pb: an X-ray spectroscopy-based analysis. *Environ. Pollut.* **2005**, *134* (2), 217−227.
(158) Semisch, A.; Ohle, J.; Witt, B.; Hartwig, A. Cytotoxicity and genotoxicity of nano - and microparticulate copper oxide: role of solubility and intracellular bioavailability. *Part. Fibre Toxicol.* **2014**, *11*, 10.
(159) Wang, S.; Liu, H.; Zhang, Y.; Xin, H. The effect of CuO NPs on reactive oxygen species and cell cycle gene expression in roots of rice. *Environ. Toxicol. Chem.* **2015**, *34* (3), 554−561.
(160) Davletova, S.; Schlauch, K.; Coutu, J.; Mittler, R. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. *Plant Physiol.* **2005**, *139* (2), 847−856.
(161) Le, C. T.; Brumbarova, T.; Ivanov, R.; Stoof, C.; Weber, E.; Mohrbacher, J.; Fink-Straube, C.; Bauer, P. Zinc finger of Arabidopsis thaliana12 (zat12) interacts with fer-like iron deficiency-induced

DOI: 10.1021/acs.est.7b04121
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

transcription factor (FIT) linking iron deficiency and oxidative stress responses. *Plant Physiol.* **2016**, *170* (1), 540−557.
(162) Pagano, L.; Servin, A. D.; De La Torre-Roche, R.; Mukherjee, A.; Majumdar, S.; Hawthorne, J.; Marmiroli, M.; Maestri, E.; Marra, R. E.; Isch, S. M.; Dhankher, O. P.; White, J. C.; Marmiroli, N. Molecular Response of Crop Plants to Engineered Nanomaterials. *Environ. Sci. Technol.* **2016**, *50* (13), 7198−7207.
(163) Pagano, L.; Pasquali, F.; Majumdar, S.; De la Torre-Roche, R.; Zuverza-Mena, N.; Villani, M.; Zappettini, A.; Marra, R. E.; Isch, S. M.; Marmiroli, M.; Maestri, E.; Dhankher, O. P.; White, J. C.; Marmiroli, N. Exposure of Cucurbita pepo to binary combinations of engineered nanomaterials: physiological and molecular response. *Environ. Sci.: Nano* **2017**, *4*, 1579−1590.
(164) Wang, Z.; Xu, L.; Zhao, J.; Wang, X.; White, J. C.; Xing, B. CuO Nanoparticle Interaction with Arabidopsis thaliana: Toxicity, Parent-Progeny Transfer, and Gene Expression. *Environ. Sci. Technol.* **2016**, *50* (11), 6008−6016.
(165) Nair, P. M.; Chung, I. M. Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignificaion, and molecular level changes. *Environ. Sci. Pollut. Res.* **2014**, *21* (22), 12709−12722.
(166) Marmiroli, M.; Imperiale, D.; Pagano, L.; Villani, M.; Zappettini, A.; Marmiroli, N. The Proteomic Response of Arabidopsis thaliana to Cadmium Sulfide Quantum Dots, and Its Correlation with the Transcriptomic Response. *Front. Plant Sci.* **2015**, *6*, 1104.
(167) Herbette, S.; Taconnat, L.; Hugouvieux, V.; Piette, L.; Magniette, M. L.; Cuine, S.; Auroy, P.; Richaud, P.; Forestier, C.; Bourguignon, J.; Renou, J. P.; Vavasseur, A.; Leonhardt, N. Genome-wide transcriptome profiling of the early cadmium response of Arabidopsis roots and shoots. *Biochimie* **2006**, *88* (11), 1751−1765.
(168) Howden, R.; Cobbett, C. S. Cadmium-Sensitive Mutants of Arabidopsis thaliana. *Plant Physiol.* **1992**, *100* (1), 100−107.
(169) Zhao, L.; Peng, B.; Hernández-Viezcas, J. A.; Rico, C.; Sun, Y.; Peralta-Videa, J. R.; Tang, X.; Niu, G.; Jin, L.; Varela-Ramirez, A.; Zhang, J. Y.; Gardea-Torresdey, J. L. Stress response and tolerance of Zea mays to CeO2 nanoparticles: cross talk among H2O2, heat shock protein, and lipid peroxidation. *ACS Nano* **2012**, *6* (11), 9615−9622.
(170) Faisal, M.; Saquib, Q.; Alatar, A. A.; Al-Khedhairy, A. A.; Hegazy, A. K.; Musarrat, J. Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. *J. Hazard. Mater.* **2013**, *250*−*251*, 318−332.
(171) Shaw, A. K.; Hossain, Z. Impact of nano-CuO stress on rice (Oryza sativa L.) seedlings. *Chemosphere* **2013**, *93* (6), 906−915.
(172) Thwala, M.; Musee, N.; Sikhwivhilu, L.; Wepener, V. The oxidative toxicity of Ag and ZnO nanoparticles towards the aquatic plant Spirodela punctuta and the role of testing media parameters. *Environ. Sci. Process. Impacts* **2013**, *15* (10), 1830−1843.
(173) Gill, S. S.; Tuteja, N. Cadmium stress tolerance in crop plants: probing the role of sulfur. *Plant Signaling Behav.* **2011**, *6* (2), 215−222.
(174) Ruotolo, R.; Peracchi, A.; Bolchi, A.; Infusini, G.; Amoresano, A.; Ottonello, S. Domain organization of phytochelatin synthase: functional properties of truncated enzyme species identified by limited proteolysis. *J. Biol. Chem.* **2004**, *279* (15), 14686−14693.
(175) Dong, C. J.; Liu, J. Y. The Arabidopsis EAR-motif-containing protein RAP2.1 functions as an active transcriptional repressor to keep stress responses under tight control. *BMC Plant Biol.* **2010**, *10*, 47.
(176) Liu, D.; Wang, X.; Lin, Y.; Chen, Z.; Xu, H.; Wang, L. The effects of cerium on the growth and some antioxidant metabolisms in rice seedlings. *Environ. Sci. Pollut. Res.* **2012**, *19* (8), 3282−3291.
(177) Trujillo-Reyes, J.; Majumdar, S.; Botez, C. E.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Exposure studies of core-shell Fe/Fe(3)O(4) and Cu/CuO NPs to lettuce (Lactuca sativa) plants: Are they a potential physiological and nutritional hazard? *J. Hazard. Mater.* **2014**, *267*, 255−263.
(178) Hong, J.; Rico, C. M.; Zhao, L.; Adeleye, A. S.; Keller, A. A.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Toxic effects of copper-based nanoparticles or compounds to lettuce (Lactuca sativa) and alfalfa (Medicago sativa). *Environ. Sci. Process. Impacts* **2015**, *17* (1), 177−185.
(179) Vittori Antisari, L.; Carbone, S.; Gatti, A.; Vianello, G.; Nannipieri, P. Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide (CeO(2), Fe(3)O(4), SnO(2), TiO(2)) or metallic (Ag, Co, Ni) engineered nanoparticles. *Environ. Sci. Pollut. Res.* **2015**, *22* (3), 1841−1853.
(180) Zuverza-Mena, N.; Armendariz, R.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Effects of Silver Nanoparticles on Radish Sprouts: Root Growth Reduction and Modifications in the Nutritional Value. *Front. Plant Sci.* **2016**, *7*, 90.
(181) Nair, P. M.; Chung, I. M. A mechanistic study on the toxic effect of copper oxide nanoparticles in soybean (Glycine max L.) root development and lignification of root cells. *Biol. Trace Elem. Res.* **2014**, *162* (1−3), 342−352.
(182) Bari, R.; Jones, J. D. Role of plant hormones in plant defence responses. *Plant Mol. Biol.* **2009**, *69* (4), 473−488.
(183) Jaillais, Y.; Chory, J. Unraveling the paradoxes of plant hormone signaling integration. *Nat. Struct. Mol. Biol.* **2010**, *17* (6), 642−645.
(184) Muday, G. K.; Rahman, A.; Binder, B. M. Auxin and ethylene: collaborators or competitors? *Trends Plant Sci.* **2012**, *17* (4), 181−195.
(185) De Smet, I.; Signora, L.; Beeckman, T.; Inze, D.; Foyer, C. H.; Zhang, H. An abscisic acid-sensitive checkpoint in lateral root development of Arabidopsis. *Plant J.* **2003**, *33* (3), 543−555.
(186) Mirzajani, F.; Askari, H.; Hamzelou, S.; Farzaneh, M.; Ghassempour, A. Effect of silver nanoparticles on Oryza sativa L. and its rhizosphere bacteria. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 48−54.
(187) Song, U.; Jun, H.; Waldman, B.; Roh, J.; Kim, Y.; Yi, J.; Lee, E. J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO2 and Ag on tomatoes (Lycopersicon esculentum). *Ecotoxicol. Environ. Saf.* **2013**, *93*, 60−67.
(188) Govorov, A. O.; Carmeli, I. Hybrid structures composed of photosynthetic system and metal nanoparticles: plasmon enhancement effect. *Nano Lett.* **2007**, *7* (3), 620−625.
(189) Haider, S.; Pal, R. Integrated analysis of transcriptomic and proteomic data. *Curr. Genomics* **2013**, *14* (2), 91−110.
(190) Zhao, L.; Hu, J.; Huang, Y.; Wang, H.; Adeleye, A.; Ortiz, C.; Keller, A. A. (1)H NMR and GC-MS based metabolomics reveal nano-Cu altered cucumber (Cucumis sativus) fruit nutritional supply. *Plant Physiol. Biochem.* **2017**, *110*, 138−146.
(191) Večeřová, K.; Večeřa, Z.; Dočekal, B.; Oravec, M.; Pompeiano, A.; Tříska, J.; Urban, O. Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles. *Environ. Pollut.* **2016**, *218*, 207−218.
(192) Meredith, A. N.; Harper, B.; Harper, S. L. The influence of size on the toxicity of an encapsulated pesticide: a comparison of micron- and nano-sized capsules. *Environ. Int.* **2016**, *86*, 68−74.
(193) Dasgupta, N.; Ranjan, S.; Ramalingam, C. Applications of nanotechnology in agriculture and water quality management. *Environ. Chem. Lett.* **2017**, *15*, 591.
(194) Singh, A.; Singh, N. B.; Afzal, S.; Singh, T.; Hussain, I. Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. *J. Mater. Sci.* **2018**, *53* (1), 185−201.
(195) Pabbi, M.; Kaur, A.; Mittal, S. K.; Jindal, R. A surface expressed alkaline phosphatase biosensor modified with flower shaped ZnO for the detection of chlorpyrifos. *Sens. Actuators, B* **2018**, *258*, 215−227.
(196) Walker, G. W.; Kookana, R. S.; Smith, N. E.; Kah, M.; Doolette, C. L.; Reeves, P. T.; Lovell, W.; Anderson, D. J.; Turney, T. W.; Navarro, D. A. Ecological Risk Assessment of Nano-enabled Pesticides: A Perspective on Problem Formulation. *J. Agric. Food Chem.* **2017**, DOI: 10.1021/acs.jafc.7b02373.
(197) Gogos, A.; Knauer, K.; Bucheli, T. D. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J. Agric. Food Chem.* **2012**, *60* (39), 9781−9792.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX