Title

Omics to address the opportunities and challenges of nanotechnology in agriculture

Journal

Critical Reviews in Environmental Science and Technology, 51(22)

ISSN

1064-3389

Authors

Majumdar, Sanghamitra

Keller, Arturo A

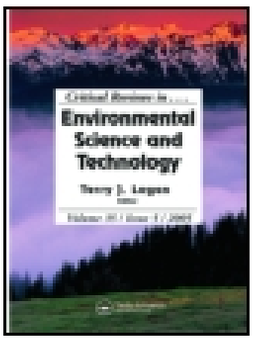
Publication Date

2021-11-17

DOI

10.1080/10643389.2020.1785264

Peer reviewed



Taylor & Francis
Taylor & Francis Group

Critical Reviews in Environmental Science and Technology

ISSN: 1064-3389 (Print) 1547-6537 (Online) Journal homepage: https://www.tandfonline.com/loi/best20

Omics to address the opportunities and challenges of nanotechnology in agriculture

Sanghamitra Majumdar & Arturo A. Keller

To cite this article: Sanghamitra Majumdar & Arturo A. Keller (2020): Omics to address the opportunities and challenges of nanotechnology in agriculture, Critical Reviews in Environmental Science and Technology, DOI: 10.1080/10643389.2020.1785264

To link to this article: https://doi.org/10.1080/10643389.2020.1785264

CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY
https://doi.org/10.1080/10643389.2020.1785264

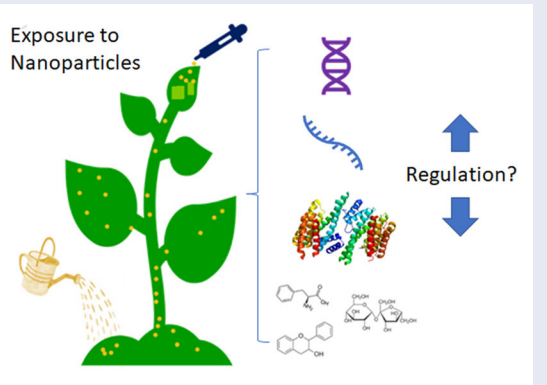
Taylor & Francis
Taylor & Francis Group

Omics to address the opportunities and challenges of nanotechnology in agriculture

Sanghamitra Majumdar[a] and Arturo A. Keller[b]

[a]Bren School of Environmental Science and Management, University of California, Santa Barbara, California, USA; [b]Center for Environmental Implications of Nanotechnology, University of California, Santa Barbara, California, USA

ABSTRACT



Increasing global food demand, and risks associated with climate change and agrochemicals demand novel and sustainable agricultural practices to improve crop yield and quality. Various strategies using nanotechnology have been explored widely to provide solutions. However, their application in the food industry is constrained by limited understanding of nanomaterial safety. Growing interest in the potential of engineered nanomaterials (ENMs) in agricultural applications has also resulted in increased crop exposure, leading to unknown risks to plants, animals and humans. Recently, considerable research has been carried out on overt plant response to ENMs; however, conclusive mechanistic information is lacking. With advancements in hyphenated analytical techniques, research on ENM-biota interactions has witnessed a paradigm shift from low-throughput, single end-point bioassays to high-throughput, discovery-oriented omic tools, including transcriptomics, proteomics and metabolomics. This review summarizes the pioneering studies utilizing omics in plants exposed to ENMs to explain phenotypic expressions and elucidate associated biological pathways. Advantages and challenges of current analytical techniques employed in proteomics and metabolomics are also discussed. We acknowledge the need for integration and application of multiomics to identify sensitive biomarkers in plants in response to ENMs, and provide mechanistic insights, in order to design enhanced and safer nano-enabled products for agriculture.

KEYWORDS Nanotechnology; metabolomics; multi-omics integration; nanotoxicology; proteomics; sustainable agriculture

CONTACT Arturo A. Keller arturokeller@ucsb.edu Center for Environmental Implications of Nanotechnology, University of California, Santa Barbara, CA 93106, USA.

1. Background

During the last decade, nanotechnology has been at the frontier of rapid advancements in different sectors such as medicine, electronics, energy production and catalysis. In the light of the daunting challenges to meet the increasing food demand for a projected global population of 9.8 billion by 2050 (FAO, IFAD, UNICEF, WFP, & WHO 2019), nanotechnology has received considerable attention as a potential solution to major challenges in agriculture and food industry (He et al., 2019; Rodrigues et al., 2017; Yin et al., 2018). Major approaches toward attaining food security as per the 2030 Agenda for Sustainable Development by United Nations include supporting interventions, aimed at developing crop resilience and adaptation to environmental variations, optimizing resource-use-efficiency by precision farming, minimizing yield gaps caused due to topographical, biophysical or socioeconomic constraints, and adopting efficient food storage and recycling processes to minimize wastage (FAO, IFAD, UNICEF, WFP, & WHO 2019). The unique physicochemical properties of engineered nanoscale materials (ENMs) are being widely studied in precision agriculture to (i) increase crop productivity and nutritional quality (nano-fertilizers) (Zulfiqar et al., 2019), (ii) targeted delivery and controlled release of agrochemicals (Guo et al., 2018; Walker et al., 2018), (iii) protect crops against pathogens (nano-pesticides) (Elmer et al., 2018; Kah et al., 2019), (iv) enhance resilience to extreme environmental conditions (Djanaguiraman et al., 2018; Jacobson et al., 2018), and (v) monitor soil/water quality and detect biotic/abiotic stressors using sensitive electrochemical devices (nano-sensors) (Giraldo et al., 2019; Mufamadi & Sekhejane, 2017; Srivastava et al., 2018) (Figure 1).

A wide range of nano-formulations such as hydroxyapatite, nano-clay, *n*Cu, *n*CuO, *n*$Cu(OH)_2$, *n*SiO_2, *n*Mn, *n*ZnO, *n*Fe_3O_4, *n*CeO_2, *n*MoO_3, carbon nanotubes, and fullerenes, are under consideration for promoting plant growth or for enhanced delivery of macro- and micro-nutrients to plant tissues (He et al., 2019; Kah et al., 2018; Rastogi et al., 2019; Yin et al., 2018). Nano-clays and -zeolites (crystalline aluminosilicates) are potential candidates for controlled release of water and agrochemicals, as well as soil and wastewater remediation (Pham et al., 2016; Yuvaraj & Subramanian, 2018). In nano-enabled pesticides, ENMs are either used as carriers (polymeric nanoparticles, *n*SiO_2, graphene oxide) for targeted and/or controlled release of conventional active ingredients, or as an active ingredient themselves (e.g. *n*TiO_2, *n*Ag, Al-based ENMs, Cu-based ENMs) (Elmer et al., 2018; Rastogi et al., 2019; Yin et al., 2018). Kah et al. carefully analyzed the benefits and gaps in the application of nano-formulations of agrochemicals, compared to conventional non-nano formulations (Kah et al., 2018). The nano-formulations are shown to enhance the efficacy of the active

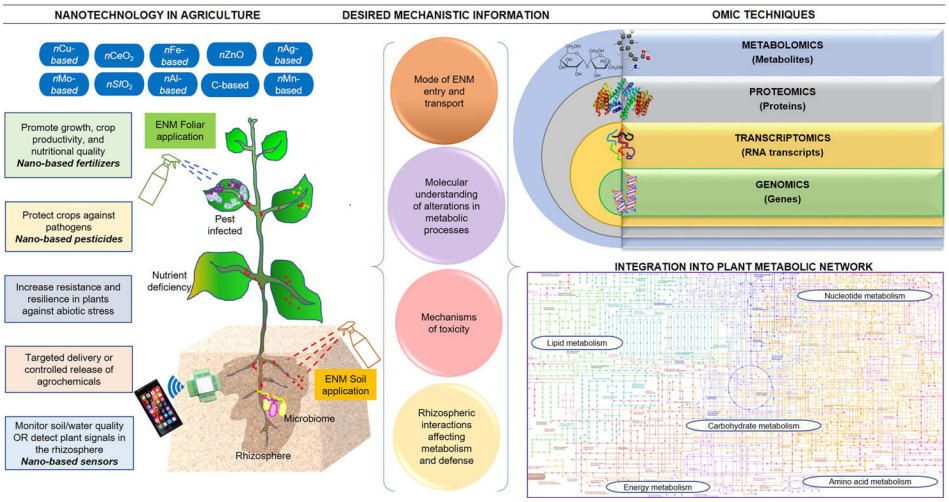


Figure 1. Application of omics to probe interaction between agricultural crops and engineered nanomaterials (ENMs).

ingredients by modifying their solubility, rate of release, bioavailability, or stability in the matrix (Kah et al., 2018, 2019). In addition to their benefits to the targeted crop, unwarranted exposure to non-targeted organisms and leaching into environmental waters can also be significantly minimized. However, the lack of mechanistic understanding of uptake, mobilization and biological response of ENMs in plants has constrained optimal utilization of nanotechnology in agricultural applications. Plants' response to ENMs is highly varied and dependent on environmental conditions, species and age, exposure dose and duration, and ENM properties, like composition, size, surface coating, morphology, stability, or dissolution, among other factors (Ma et al., 2018). Researchers have employed advanced electron microscopy, synchrotron-based imaging techniques and single particle-inductively coupled plasma mass spectrometry (*sp*-ICP-MS) to locate ENMs in plant tissues upon foliar or root exposure (Avellan et al., 2019; Castillo-Michel et al., 2017; Keller et al., 2018). Such studies have effectively proved that some ENMs enter through roots or leaves and can cross biological barriers to transport within plants, unlike their conventional analogues (Ma et al., 2015; Sanzari et al., 2019). But the molecular drivers of the uptake and transport of ENMs in plants remains inconclusive.

The tunable surface properties and size compatibility of ENMs with biomolecules and cellular pores are currently harnessed for agricultural applications; however, these properties may also render the plant and associated microbial community vulnerable to toxicity. In addition to their intentional use in agriculture, ENMs from industrial processes and consumer products also inadvertently enter the wastewater system and bioaccumulate in biosolids which eventually may find their way to agricultural fields (Cornelis

et al., 2014; Garner et al., 2017). By 2022, the global market revenue for ENMs is estimated to escalate to $16.8 billion from $7.3 billion in 2016 (He et al., 2018), but a comprehensive understanding of their long-term impact on human health and the environment is still lacking (Giese et al., 2018; He et al., 2018). In most publications, the impact of ENMs in agricultural applications are reported in terms of nutrient accumulation in tissues, plant growth and yield, or effect on the onset of pathogen infection (Dimkpa et al., 2018; Elmer et al., 2018). Studies on ENM exposure in agricultural crops in hydroponic, greenhouse or field trials have been conducted for short-term as well as full life cycle (Barrios et al., 2016; Du et al., 2015; Ma et al., 2016; Majumdar et al., 2015; Rawat et al., 2018; Zhao et al., 2015), entailing transfer along terrestrial food webs (Majumdar, Trujillo-Reyes, et al., 2016; Unrine et al., 2012). Studies have reported varied effects in agricultural crops in response to ENMs, depending on their size, type, concentrations, surface properties, route of exposure, growth media and plant species (Conway et al., 2015; Ma et al., 2015; Majumdar et al., 2014; Nhan et al., 2015; Rico et al., 2015). The parameters investigated in most of the ENM toxicological assessment studies in plants are however limited to basic agronomic traits and accumulation of the constituent metal/metalloid in the tissues (Dimkpa et al., 2018). Comparative assessment of metabolic activities in ENM-exposed and untreated plants have been reported in several nanotoxicity studies. Plant metabolic responses were mostly evaluated based on photosynthetic and gas exchange measurements, macro- or micro-nutrient contents, and indirect biochemical analysis for oxidative stress (Conway et al., 2015; Majumdar, Peralta-Videa, et al., 2016; Majumdar et al., 2014; Pérez-Labrada et al., 2019; Rabêlo et al., 2019; Rawat et al., 2018; Rico et al., 2013, 2015). Although these studies provide insights on plant's health, they do not provide a holistic and detailed understanding of the regulatory mechanisms involved in cellular response.

1.1. Adopting "omics" to study interactions at ENM-plant interface

A comprehensive understanding of the biological networks at multiple levels is crucial to harness the full potential of ENMs for sustainable food production (Yin et al., 2018). In recent years, probing of ENM-plant interaction have evolved from traditional, single endpoint assays to discovery oriented, high-throughput system biology approaches, referred as "omics". This is supported by advancements in the sensitivity and accuracy of analytical techniques and bioinformatic tools (Quanbeck et al., 2012). The suffix "omics" refers to unbiased screening of biomolecules in an organism, specifically genes (*genomics*), mRNA (*transcriptomics*), proteins

(*proteomics*), or metabolites (*metabolomics*) (Figure 1). Systems biology approach has been implemented to decode the molecular mechanisms in plants and elucidate the behavior of genes, proteins and metabolites in response to biotic or abiotic stressors (Kumar et al., 2015). With the emerging need for mechanistic understanding of complex agronomic traits and crops' response to ENM exposure, omic technologies have gained momentum in precision agriculture and nanotoxicity studies. This paradigm helps in generating hypotheses by monitoring response of biomolecules upon systematically perturbing biological processes with ENMs, followed by integration of global datasets onto pathways using advanced bioinformatics algorithms (Majumdar et al., 2019; Ruotolo et al., 2018). Realization of the underlying molecular mechanisms in plants will provide cues for designing ENMs for specific applications like increasing resilience to pests or environmental conditions, targeted delivery of nutrients or pesticides, stimuli-responsive agrochemical release, or ENM-enabled biosensing. The sensitivity of omic techniques allows to capture, quantitate and distinguish the cellular and molecular level changes in an organism when exposed to ionic, nano- or bulk- form of any particle of interest at considerably lower and environmentally realistic doses; these deductions are not obvious from phenotypic responses or less sensitive biochemical assays (Majumdar et al., 2019). These techniques also allow to compare responses at multiple hierarchical levels across different plant species, age, growth/environmental conditions, and ENM exposures.

In plants, transcriptomics has been the most applied omic technique, used to identify the transcription factors as predictive biomarkers of ENM toxicity (Ruotolo et al., 2018), which are correlated to phenotypic responses. However, this bottom-up approach based on upward chain of causality has several constraints that result in inconclusive nature of such approach (do Amaral & Souza, 2017). These constraints emerge from the uncertainty resulting from posttranscriptional processes, posttranslational protein modifications, and stimulus-induced metabolite level changes. The higher level of organization representing the metabolome or proteome in an organism are not fully determined by the properties of the lower levels (genes); instead, they regulate the functionality of lower levels in a downward causation chain in response to stimuli (do Amaral & Souza, 2017). Metabolomic analyses allow functional annotation of uncharacterized genes or proteins, thereby filling knowledge gaps in plant metabolic machinery (Hegeman, 2010; Quanbeck et al., 2012). In addition, metabolomics does not depend on the data generated from model plant species, hence could be easily applied to model as well as non-model species (Matich et al., 2019). Thus, due to the exploratory nature of ENM-plant interaction studies, it is recommended to follow the downward causation approach that

correlates phenotypic expression with the metabolome of plants, which can complement proteomic and transcriptomic profiles in a pathway analysis network. This review consolidates the pioneering studies in plant metabolomics and proteomics, intended to gain insights into ENM-plant interactions. Studies employing other omic tools have been discussed briefly. We discuss novel analytical platforms employed in metabolomics and proteomics in plants in response to ENMs and address the important factors in such analyses. Finally, we postulate the vulnerable biological pathways in plants in response to ENMs and how integration of multiomic datasets can be exploited to address major mechanistic concerns and enable realization of wider application of nanotechnology in agriculture.

2. Metabolomics: linking molecular events to ENM-associated phenotype

Metabolites are the end-product of cellular regulatory processes that reflect the ultimate response of an organism to any external stimulus (Figure 1). The plant metabolic pathway databases, curated from experimental literature by the Plant Metabolic Network (PMN 13.0, Carnegie Institution for Science), report 4,544 compounds involved in 1,123 pathways across 350 plant species (Schläpfer et al., 2017). Plants collectively produce a diverse array of $\geq$ 200,000 metabolites, which are broadly divided into two major categories, primary and secondary metabolites (Dixon, 2001). Primary metabolites, which include carbohydrates, amino acids, vitamins, organic acids, and fatty acids, are required for plant growth and development (Fiehn, 2002). Secondary metabolites are synthesized from the primary metabolites for adaptation and defense response in plants (Fiehn, 2002). The major classes of secondary metabolites are polyketides, terpenoids, steroids, phenylpropanoids, alkaloids, and glucosinolates, which have their own biogenetic pathways and thousands of products and pathway intermediates (Hounsome et al., 2008). Primary metabolites are universal and conserved in their structures throughout the plant kingdom, whereas the secondary metabolites are species-specific and differ in chemical complexity.

Any alteration in plant physiology in response to a xenobiotic, such as ENMs, is regulated by molecular events and is reflected at the level of metabolites that participate in interconnected biological pathways such as glycolysis, citric acid cycle, gluconeogenesis, biosynthesis of amino acids, biosynthesis of secondary metabolites, nitrogen metabolism, and fatty acid metabolism. Plant roots also exude metabolites as signaling molecules to defend or adapt to stressors as well as modulate soil chemistry and/or biochemical pathways to influence nutrient bioavailability (Mhlongo et al., 2018). Plant and rhizosphere metabolomics thus provide a snapshot of the

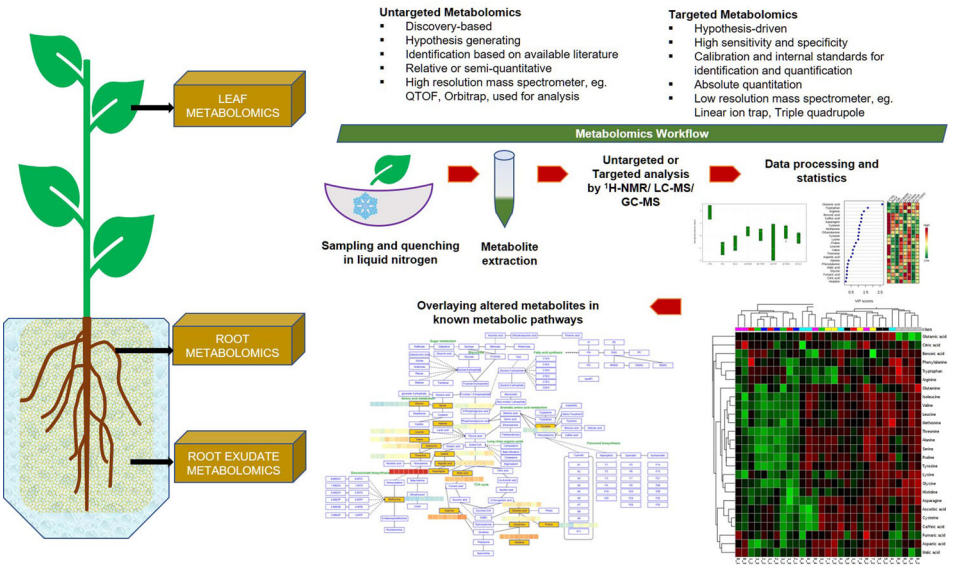


Figure 2. Metabolomic analysis in plants exposed to ENMs.

entire plant-associated metabolome, that can be directly correlated with real-time functional footprint of the cellular state. The need for measuring metabolite levels in plants in response to ENMs was realized early on by the nanotoxicology community. However, most studies evaluated the total content of classes of metabolites such as sugars, phenolics, flavonoids, chlorophylls, non-enzymatic antioxidants, lignin, etc. using less sensitive biochemical assays (Majumdar, Peralta-Videa, et al., 2016; Pérez-Labrada et al., 2019; Rabêlo et al., 2019; Rico et al., 2013). In some studies, profiling of specific classes of compounds like carbohydrates, fatty acids, and amino acids was also performed (Majumdar et al., 2019; Rico et al., 2015, 2013). However, based on the evaluation of a few metabolic parameters, a comprehensive understanding of the underlying mechanisms of ENM transport and effects is rather limited and biased by author's interpretations. To address these challenges and identify the crucial role of the affected metabolites in response to ENMs, it is important to increase the analytical coverage of the plant metabolome.

2.1. Analytical techniques and challenges in plant metabolomics

Metabolomic analysis can be categorized as untargeted or targeted, the choice of which depends on the scope of the study (Figure 2). Key factors that separate untargeted from targeted metabolomics are: (a) extent of sample preparation required; (b) number of metabolites detected; (c) level of quantification; and (d) extent of data processing postacquisition. Untargeted metabolomics is a discovery-based approach that screens the

entire pool of metabolites in an organism. In nanotoxicity studies, untargeted metabolomics can be used to discover unknown metabolites of significance and identify markers in order to generate hypothesis on biological pathways involved in response to ENM exposures (Piasecka et al., 2019). Metabolite identification is based on available literature or in-house experimental database, and the quantitation is either relative or semi-quantitative, aided with extensive data processing (Piasecka et al., 2019). However, untargeted metabolomics also suffers from practical challenges. Plant metabolites span over a broad range of composition and physio-chemical properties, which makes it challenging to extract and identify all of them simultaneously with acceptable recovery using a single analytical procedure. In addition, low molecular weight plant metabolites differ by several orders of magnitude, ranging from femto- or picomolar (hormones) to millimolar concentrations (organic acids, sugars), which present sensitivity and accuracy challenges for the detection of less abundant metabolites (Piasecka et al., 2019). Targeted metabolomics, on the other hand, focus on selected classes of chemically characterized and biologically annotated metabolites. Targeted metabolomics is hypothesis-driven, in which a defined set of known metabolites are analyzed with significantly higher selectivity and sensitivity, focusing on a specific biochemical question. Calibration and isotopically-labelled internal standards are used for absolute quantitation of the metabolites under investigation.

Metabolomic analysis demands careful attention to details at each analytical step including sampling, metabolite extraction, storage, instrumental analysis, data processing, and interpretation for factual representation and reproducibility (Figure 2). Metabolite levels in plants are highly sensitive to growth and environmental conditions. Hence, a robust experimental design, randomization, efficient reporting of experimental details as per the Metabolomics Standards Initiative guidelines, and submission of results in data repositories are highly advisable, to ensure reproducibility and consistency of the metadata obtained from metabolomic analysis (Fiehn et al., 2007). To compensate for the qualitative and quantitative variations among plant samples, biological replicates are essential, which results in more powerful statistical analysis. However, in case of high variability and limited sample availability, sample pooling is a common procedure to represent the population, which nonetheless should be reported and considered during data analysis (Rodrigues et al., 2019). Although these requirements are quintessential for any metabolomic experiment, nanotoxicity studies should also consider ENM stability in the exposure media throughout the experiment duration, environmentally or agriculturally relevant dosing, ambient fluctuations, use of appropriate positive and negative controls, and comparison with ionic and bulk-particle controls to identify nano-specific effects. Representative techniques and key challenges

in each step of the metabolomic analysis in ENM-plant interaction research are summarized below (Figure 2).

2.1.1. Sampling, quenching, and extraction

In a metabolomic study, a robust sampling protocol is critical in order to obtain maximum information from the plants exposed to ENMs. Some of the factors considered during sampling are: scope of the study, route of exposure (via roots or leaves), kinetic of ENM transport in the species under investigation, growth stage of the plant and time of the day when harvesting is done. Different plant organs such as root, leaf or fruit have distinct metabolic footprint owing to varying cellular organization. Thus, to prevent loss of information about chemical signaling across organs, it is necessary to analyze them separately. The metabolite profile of a tissue also varies significantly depending on the growth stage of the plant; hence, results from an ENM exposure study in a germinating seedling cannot be extended to compare or interpret metabolic events in the plant in its vegetative or matured stage, or vice versa.

Tissues collected from untreated and ENM-treated plants should be immediately quenched in liquid nitrogen or freeze-dried to capture the metabolic state of the plant at the desired moment. Prior to freezing, tissues may be washed with water at room temperature, if necessary; cold or hot solvents must be avoided to prevent leakage of intracellular metabolites. Frozen tissues are ground to homogenous dry powder using precooled homogenizing apparatus, and appropriate extraction methods must be used depending on the analytical technique used. To prevent loss of unstable metabolites and to maximize analytical recovery through all the steps of extraction, adequate sample storage and handling is important. In untargeted analysis, the aim is to broaden the coverage of metabolites (polar and non-polar) and maximize identification. The most common strategy is to extract a wide range of metabolites in solvent mixtures, such as methanol/methyl tert-butyl ether/water or methanol/chloroform/water, followed by separation of the polar and non-polar metabolites by biphasic partitioning (Matich et al., 2019). Each phase is collected for analysis using suitable analytical platforms. In targeted analysis, specific extraction protocols are optimized to retain the metabolites of interest. Additional sample processing such as filtration or solid phase extraction are also used to reduce matrix effects and remove undesired metabolites.

2.1.2. Data acquisition

The most common analytical platforms used in plants metabolomics are nuclear magnetic resonance (NMR) and single- or tandem-mass spectrometry

(MS or MS/MS) hyphenated with chromatographic separation techniques, for example, liquid chromatography (LC) and gas chromatography (GC) (Matich et al., 2019). NMR allows structure elucidation of metabolites and detailed analysis of the biomolecular composition of plant extracts with relatively simple sample preparation and quantitative comparison of analytes (Lu et al., 2017). However, due to poor sensitivity and dynamic range, NMR-based metabolomics can only detect abundant metabolites, present in millimolar levels (Kim et al., 2010; Zhao, Hu, Huang, Wang, et al., 2017; Zhao, Huang, Hu, et al., 2016). MS-based techniques that monitor mass-to-charge ratios (m/z) of all ionizable molecules in a sample, are however becoming the method of choice due to their higher sensitivity, reproducibility, ease of hyphenation with separation techniques and control over the method of ionization of the analytes (Lu et al., 2017). GC-MS has been the most common metabolomic technique in ENM-plant interaction studies, primarily due to the ease of use, high chromatographic resolution, low limits of detection and quantitation, and the availability of extensive database for identification (Rico et al., 2020; Wu et al., 2017; Zhang et al., 2018; Zhao, Hu, Huang, Wang, et al., 2017; Zhao, Hu, Huang, Fulton, et al., 2017; Zhao, Huang, et al., 2017; Zhao, Huang, Hannah-Bick, et al., 2016; Zhao, Huang, Hu, et al., 2016; Zhao, Huang, & Keller, 2018; Zhao, Huang, Paglia, et al., 2018; Zhao, Huang, Zhou, et al., 2016; Zhao, Ortiz, et al., 2016; Zhao, Zhang, Wang, et al., 2019; Zhao, Zhang, White, et al., 2019; Zhao et al., 2018). However, GC-MS is best suitable for volatile and thermally stable metabolites, and requires derivatization/chemical modification of polar metabolites such as sugars, amino acids and organic acids (Lu et al., 2017). In contrast, LC-MS allows analysis of polar and high molecular weight metabolites without any derivatization. For untargeted analysis, LC is hyphenated with high resolution MS like time-of-flight (qTOF) or Orbitrap mass analyzers with electrospray ionization (ESI). Nevertheless, LC-MS also suffers from drawbacks due to ion suppression, differential adduct formation and retention time shifts due to matrix effects. Different analytical approaches are however complementary, and can be used together to obtain a comprehensive coverage of metabolites with high confidence. Extraction of plant metabolites in solvent mixtures results in an immediate loss of inter- and sub-cellular resolution. Advances in MS imaging (MSI) techniques (Mhlongo et al., 2018) have made it possible to map metabolites in intact plant tissues at sub-cellular level and connect the spatial complexity of the plant molecular organization to phenotypical features. Some techniques that have been hyphenated with MSI for imaging metabolites in plant specimens are matrix-assisted laser desorption/ionization (MALDI) and laser ablation electrospray ionization (LAESI) (Etalo et al., 2015; Nakabayashi et al., 2019). However, these approaches have not yet been utilized in ENM-plant interaction studies.

In targeted metabolomics, low resolution MS such as linear ion traps or triple quadrupole (QqQ) are predominantly used (Lu et al., 2017; Matich et al., 2019). Advances in QqQ in single reaction monitoring (SRM) mode allows robust absolute quantification of metabolites with high sensitivity and selectivity, even at trace levels with relatively high throughput. Column chemistry and its retention mechanism are extremely critical for targeted analysis of metabolites using LC-MS; and hence, reverse phase (RP) and hydrophilic interaction liquid chromatography (HILIC) are used to cover broad range of metabolites with contrasting polarity (Huang et al., 2018, 2019; Jorge et al., 2016). Targeted analysis is particularly useful as a follow up from untargeted analysis, in order to focus on specific metabolic pathways or testing application of ENMs in pathogen defense, nutrient acquisition, photosynthetic efficiency or productivity.

2.1.3. Data processing and interpretation

Data processing in metabolomics comprises baseline correction, feature extraction, spectral alignment across samples, identification and interpretation (Piasecka et al., 2019) (Figure 2). Post data-acquisition, baseline correction is performed on the datasets to remove low frequency artifacts and aberrations across multiple samples that could be generated by experimental or instrumental variation. As chromatographic retention time may vary between samples, spectral alignment is performed across the dataset, which may precede or follow feature extraction process. In MS-based studies, peak-based algorithms are used to extract and quantify all relevant chromatographic and spectral information for all known and unknown metabolites in each sample. Upon detection, related ions indicative of a single-component chromatographic peak (adducts or multiple-charged) are identified and grouped (Perez de Souza et al., 2019). Several open source and commercial MS reference and MS/MS library matching databases are available; but the databases on plant metabolites is limited and they are not fully optimized for all analytical platforms. Electron ionization fragmentation patterns and indexed retention times for an extensive array of compounds using GC-MS and NMR spectral library are available; however, LC-MS data lack standardization in the available resources (Hegeman, 2010). However, due to an increasing number of studies in plant metabolism, high quality spectral and chromatographic data are continuously being added to and curated within these spectral libraries, which will eventually improve the routine peak identification in non-model plants. Software such as XCMS, PRIMe, MeltDB etc. are used for data processing including feature detection, retention time correction, alignment, annotation, statistical analysis, and data visualization. Another online tool, MetaboAnalyst, is used to process identified metabolites for data normalization, statistical

analysis, and visualization (Chong et al., 2018). Statistical analysis of the metabolomics data helps to identify features that are differentially regulated compared to control samples. The identified markers are then projected on to the available metabolic networks, which can be used in a phenotypic context to generate mechanistic hypothesis.

2.2. Application of metabolomics for elucidating mechanisms of ENM-plant interactions

Exposure to ENMs elicits signaling cascades in plant metabolic pathways resulting in dynamic shifts in the production and regulation of cellular metabolites. Here, we have reviewed fifteen publications that have applied untargeted metabolomics to elucidate underlying mechanisms in plants exposed to various metal/metal oxide nanoparticles (*n*Cu, *n*$Cu(OH)_2$, *n*CeO_2, *n*Fe_3O_4, *n*TiO_2, *n*SiO_2, *n*Ag and *n*CdO,) by acute or chronic application via root or leaves (Table 1). A few studies have also employed targeted metabolomics on selected group of metabolites, based on the findings from untargeted analyses (Huang et al., 2018, 2019; Majumdar et al., 2019).

2.2.1. Copper based-NPs

In recent years, several studies have investigated the response of different crop species to Cu based-ENMs using untargeted metabolomics as summarized in Table 1 (Zhao, Hu, Huang, Wang, et al., 2017; Zhao, Huang, et al., 2017; Zhao, Huang, Hannah-Bick, et al., 2016; Zhao, Huang, & Keller, 2018; Zhao, Huang, Zhou, et al., 2016; Zhao, Ortiz, et al., 2016). A week of *n*Cu (10 and 20 mg/l) root exposure to cucumber (*Cucumis sativus*) plants grown in hydroponic growth media, altered the accumulation of at least 22 leaf metabolites (Zhao, Huang, Hu, et al., 2016). In this pioneering metabolomic study, ^{1}H-NMR detected increased accumulation of secondary metabolites involved in defense response and cell signaling like 4-aminobutyrate (GABA), acetylglucosamine, phenyl lactate, whereas those involved in amino acid metabolism (Phenylalanine (Phe)/Tyrosine (Tyr), Tryptophan (Trp), and Arginine (Arg)/Proline (Pro), fatty acid biosynthesis, riboflavin metabolism, and flavonoid biosynthesis were suppressed. When grown in *n*Cu spiked soil (200, 400 and 800 mg/kg) until full maturity, cucumber fruits also revealed alteration in metabolite profile which affected their nutritional quality (Zhao, Huang, Zhou, et al., 2016). Two different analytical techniques were used as complementary platforms; ^{1}H-NMR identified only ten metabolites, whereas GC-MS identified 107 metabolites. *n*Cu treatments altered fruit metabolites including carbohydrates (1-kestose, xylose, and fructose), amino acids and their derivatives (ornithine (Orn), citrulline (Cit), glycine (Gly), Pro, oxo-Pro, methionine

Table 1. Summary of metabolomic studies in plants exposed to ENMs.

ENM	Size	Hydrodynamic dia. and zeta-potential characterization in suspension	Plant species	Age at exposure (days)	Growth media	Exposure route	Exposure period	Exposure concentration	Tissue analyzed	Instrument used	Metabolic pathways affected	Reference
*n*Cu	40 nm	2590 ± 1138 nm, (-) 29.4 ± 0.8 mV	*Cucumis sativus*	14	Hydroponic	Root	7 days	10, 20 mg/l	Root exudate, Leaf	^{1}H-NMR, GC-TOF-MS	Amino acid metabolism, Fatty acid biosynthesis, Flavonoid biosynthesis Increased defense response (4-aminobutyrate, acetylglucosamine, phenyl lactate)	(Zhao, Huang, Hu, et al., 2016)
*n*Cu	40 nm	2590 ± 1138 nm, (-) 29.4 ± 0.8 mV	*Cucumis sativus*	0	Soil	Seed/ Root	68 days	200,400, 800 mg/kg	Fruit	GC-TOF-MS	Carbohydrate metabolism, TCA cycle, Amino acid metabolism, α-linolenic metabolism, Alkaloid biosynthesis, Fatty acid metabolism Increased defense response (proline, benzoic acid, alkaloids)	(Zhao, Huang, Zhou, et al., 2016)
*n*Cu(OH)$_2$, Kocide 3000	>1000 nm	1532 ± 580 nm, (-) 47.6 ± 43 mV	*Lactuca sativa*	24	Soil	Foliar	4 weeks (chronic, 2 times/week)	1050,1550, 2100 mg/l	Leaf	GC-TOF-MS	Amino acid metabolism, TCA cycle, Pantothenate and CoA biosynthesis, Glycolysis or gluconeogenesis, Pyruvate metabolism Decreased defense response (Dehydroascorbic acid, oxalic acid, threonic acid, 4-aminobutyrate, cis-caffeic acid, chlorogenic acid, 3,4-hydroxycinnamic acid)	(Zhao, Ortiz, et al., 2016)
*n*Cu(OH)$_2$, Kocide 3000	>1000 nm	1532 ± 580 nm, (-) 47.6 ± 43 mV	*Cucumis sativus*	21	Soil	Foliar	7 days (chronic, 3 times/day)	100, 1000 mg/ L (2.5 and 25 mg/plant)	Leaf	GC-TOF-MS	Amino acid (Arg/Pro) metabolism	(Zhao, Huang, Zhou, et al., 2016)
*n*Cu(OH)$_2$, Kocide 3000	>1000 nm	1532 ± 580 nm, (-) 47.6 ± 43 mV	*Zea mays*	21	Soil	Foliar	7 days (chronic, 3 times/day)	100, 1000 mg/ L (10 and 100 mg/plant)	Leaf	GC-TOF-MS	Glycolysis, TCA cycle, Shikimate pathway, Phenylpropanoid pathway	(L. Zhao, Y. Huang, & A. A. Keller, 2018
*n*Cu(OH)$_2$, Kocide 3000	>1000 nm	1532 ± 580 nm, (-) 47.6 ± 43 mV	*Spinacia oleracea*	28	Soil	Foliar	7days (chronic, 3 times/day)	100, 1000 mg/ L (1.8 and 18 mg/plant)	Leaf	GC-TOF-MS	Increased defense response Phenylpropanoid pathway, Ascorbate/ aldarate pathway, Decreased defense response (Ascorbic acid, α-tocopherol, threonic acid, 4-aminobutyrate, β-sitosterol, 4-hydroxybutyric acid, ferulic acid)	(Zhao, Huang, et al., 2017)
*n*Ag-citate or PVP coated	NR	*nAg*-citrate 72 ± 9 nm, nAg-PVP 2.6 ± 0.4 nm	Arabidopsis thaliana (Columbia-2 ecotype, wild)	7	Agar with 1/4 Hoagland solution	Foliar	1 day	1 mg/l	Leaf	LC-QTOF-MS	Fatty acids metabolism (Sphingolipids)	(Chavez Soria et al., 2017)
*n*Ag	20 nm	175 ± 5 nm, 6.2 ± 1.5 mV	*Cucumis sativus*	28	Soil	Foliar	7 days	10, 100 mg/l	Leaf	GC-MS	Carbohydrate metabolism, TCA cycle, Increased defense response (phenolic compounds, fatty acid, salicylic acid)	(Zhang et al., 2018)

(*continued*)

Table 1. Continued.

ENM	Size	Hydrodynamic dia. and zeta-potential characterization in suspension	Plant species	Age at exposure (days)	Growth media	Exposure route	Exposure period	Exposure concentration	Tissue analyzed	Instrument used	Metabolic pathways affected	Reference
$nCeO_2$	10–30 nm (spherical)	NR	*Phaseolus vulgaris*	21	Soil	Root	15 days (chronic application)	250-2000 mg/l (50 and 200 mg)	Leaf	UHPLC/ QTOF-MS	Decreased pteridine-related compounds, Increased defense response (carotenoid, phenolic compounds)	(Salehi et al., 2018)
						Foliar	15 days (chronic application)	250-2000 mg/l (50 and 200 mg)	Leaf	UHPLC/ QTOF-MS	Structural integrity: Increased membrane lipids and lignans (sesaminol-2-O-β-glucoside and diphyllin), Pteridin and lignin synthesis Decreased defense response (carotenoids, glucosinolates, terpenoids, and phenolics)	(Salehi et al., 2018)
$nCeO_2$	67 × 8 nm (rod)	NR	*Triticum aestivum*	9	Soil (low and high N supply)	Root	18 days before full harvest	500 mg/kg soil	Seed	GC-QTOF-MS	Pentose phosphate pathway, Fructose-mannose metabolism, Phosphoribosyl pyrophosphate pathway	(Rico et al., 2020)
$nSiO_2$	20 nm	876 ± 41 nm, (+) 19.5 ± 0.37 mV	Zea mays	NR	Soil	Root	28 days	100 mg/kg	Leaf, root	GC-MS	*Leaves:* Amino acid metabolism (Arg/Pro, Gly/Ser/Thr), Methane metabolism, Pantothenate and CoA biosynthesis, Carbon metabolism (TCA cycle, glycolysis, gluconeogenesis, pyruvate metabolism), Pyridine metabolism, Increased defense response (Tyr, Phe, Glu, 4-aminobutyrate, succinate semialdehyde, polyamines) *Roots:* inositol phosphate metabolism, TCA cycle	(Zhao, Zhang, White, et al., 2019)
$nTiO_2$	5–10 nm	293 ± 17 nm	Zea mays	NR	Soil	Root	28 days	100 mg/kg	Leaf, root	GC-MS	*Leaves:* Amino acid metabolism (Arg/Pro, Gly/Ser/Thr), Methane metabolism, Pantothenate and CoA biosynthesis, Carbon metabolism (TCA cycle, glycolysis, gluconeogenesis, pyruvate metabolism), Pyridine metabolism, Increased defense response (Tyr, Phe, Glu, 4-aminobutyrate, succinate semialdehyde, polyamines) *Roots:* Inositol phosphate metabolism, TCA cycle, Ascorbate/ aldarate metabolism, Methane metabolism, Glycerolipid metabolism, Glyoxylate and dicarboxylate metabolism	(Zhao, Zhang, White, et al., 2019)

(*continued*)

Table 1. Continued.

ENM	Size	Hydrodynamic dia. and zeta-potential characterization in suspension	Plant species	Age at exposure (days)	Growth media	Exposure route	Exposure period	Exposure concentration	Tissue analyzed	Instrument used	Metabolic pathways affected	Reference
$nTiO_2$	20 nm	593 ± 7 nm, (-) 21.4 ± 0.64 mV	Oryza sativa	7	Hydroponic	Root	14 days	100,200, 500 mg/l	Leaf	GC-MS	TCA cycle, Pentose phosphate pathway, Starch and Sucrose metabolism, Glyoxylate and dicaroxylate metabolism	(B. Wu et al., 2017)
nFe_3O_4	30 nm	1230 ± 56 nm, (+) 11.6 ± 0.64 mV	Zea mays	NR	Soil	Root	28 days	100 mg/kg	Leaf, root, soil	GC-MS	*Leaves:* Isoquinoline alkaloid biosynthesis, Glyoxylate and dicarboxylate metabolism, Amino acid metabolism (Arg/Pro, Gly/Ser/Thr), Methane metabolism, Pantothenate and CoA biosynthesis, Carbon metabolism (TCA cycle, glycolysis, gluconeogenesis, pyruvate metabolism), Pyridine metabolism, Increased defense response (Tyr, Phe, Glu, 4-aminobutyrate, succinate semialdehyde, polyamines) *Roots:* Inositol phosphate metabolism, TCA cycle, Ascorbate/ aldarate metabolism, Methane metabolism, Glycerolipid metabolism, Glyoxylate and dicarboxylate metabolism	(Zhao, Zhang, White, et al., 2019)
*n*CdO	7–60 nm	NR	*Hordeum vulgare*	14	Soil	Foliar	21 days	2.03 ± 0.45 × 105 particles per cm3 air	Leaf, Root	HPLC-LTQ Orbitrap HRMS for polar fractions, GCMS for non-polar fractions	*Leaves:* Amino acid metabolism (especially Phe, Trp, Val, Leu, Asn, Tyr) Afftected defense response (amino acids: Trp and Phe, phenolics: gallic acid, sinapic acid, ferulic acid) *Roots:* Amino acid metabolism (especially Phe, Trp, Val, Leu, Asn, Tyr) Carbohydrate metabolism	(Večeřová et al., 2016)
nY_2O_3	30 nm		*Zea mays*	0	Suspension	Seed	6 days	10, 500 mg/l	Seed	^{1}H-NMR	Carbohydrate metabolism, TCA cycle and amino acid metabolism	(Gong et al., 2019)
C-nanodots	3 nm	(-) 13.21 ± 1.64 mV	*Arabidopsis thaliana* (Columbia ecotype, wild)	7	Artificial medium	Root	7 days	125-1000 mg/l	Root, shoot	GC-MS	Carbohydrate metabolism, Cell wall stress, Increased defense response	(Chen et al., 2018)
C60 fullerols	500 nm	389 ± 6 nm, (-) 39.5 ± 2.5 mV	*Cucumis sativus*	21	Soil	Foliar	2 day (chronic , twice/day)	100-200 mg/l (1-2 mg/plant)	Leaf	GC-MS	Degradation of plasma membrane metabolites like linolenic and palmitic acid, Increased accumulation of antioxidant metabolites	(Zhao, Zhang, Wang, et al., 2019)

*NR- not reported.

(Met), and aspartic acid (Asp), carboxylic acids (citrate, glutarate, shikimate, benzoate and pelargonate) and fatty acids (arachidic, linolenic, and caprylic acids) (Zhao, Huang, Zhou, et al., 2016). Accumulation of Pro and benzoic acid in the fruits indicates response to oxidative stress. *n*Cu perturbed 15 biological pathways in the fruits, of which galactose metabolism and tricarboxylic acid (TCA) cycle (succinate, fumarate, and pyruvate increased; citrate decreased) were most significant. In addition, Arg and Pro metabolism, Gly/serine (Ser)/threonine (Thr) metabolism, isoleucine (Ile)/leucine (Leu)/valine (Val) metabolism, Phe/Tyr/Thr metabolism, α-linolenic metabolism, and alkaloid biosynthesis were affected, demonstrating impact on cell membrane and oxidative stress.

Kocide-3000 is a commercial micron-sized pesticide, composed of copper hydroxide ($Cu(OH)_2$) nanosheets, which can be applied using aerial or ground spray in the field. Zhao et al. investigated the metabolomes of lettuce, cucumber, maize and spinach plants chronically sprayed with Kocide (Zhao, Ortiz, et al., 2016; Zhao, Huang, et al., 2017; Zhao, Huang, & Keller, 2018). Twenty four-days-old lettuce (*Lactuca sativa*) plants were foliar sprayed with 1050, 1550, 2100 mg/l Kocide twice a week for four weeks (Zhao, Ortiz, et al., 2016). Although the chronic exposure did not induce overt response, metabolomic analysis of the leaves revealed differential accumulation of 50 metabolites ultimately affecting six biological pathways, including Gly/Se/Thr metabolism, Ala/Asp/Glu metabolism, TCA cycle, pantothenate and CoA biosynthesis, glycolysis or gluconeogenesis, and pyruvate metabolism. Chronic exposure to Kocide deteriorated the antioxidant defense mechanism in the lettuce plants expressed by decreasing levels of phenolic compounds like cis-caffeic acid, chlorogenic acid, 3,4-hydroxycinnamic acid. Dehydroascorbic acid, its oxidation products (oxalic acid and threonic acid) and GABA levels were also diminished at high exposure concentrations, suggesting impairment of the antioxidant system in lettuce. A chronic exposure of spinach (*Spinacia oleracea*) leaves for 7 days to 1000 mg/l Kocide (18 mg Kocide/plant) also resulted in depreciated levels of antioxidant or defense associated metabolites, including ascorbic acid, a-tocopherol, GABA, threonic acid, β-sitosterol, 4-hydroxybutyric acid, and ferulic acid, resulting in the perturbation of phenylpropanoid and ascorbate/aldarate pathways (Zhao, Huang, et al., 2017). However, the decreased levels of antioxidants and activation of antioxidant defense system in spinach leaves were driven by Cu ion release. In addition to amino acids (Ala, Val, Leu, Asn, Met, Asp), accumulation of phytol, a chlorophyll degradation product, was also decreased in Kocide-exposed spinach leaves, similar to Kocide-exposed maize (*Zea mays*) and cucumber leaves (Zhao, Huang, & Keller, 2018). Metabolomic response in artificial soil-grown three-weeks old cucumber and maize plants exposed to 100 and 1000 mg/l

Kocide by foliar spray for a week was compared; however it is to be noted that the final dose in maize plants (10 and 100 mg/plant) were higher than the cucumber plants (2.5 and 25 mg/plant) due to hydrophobicity of maize leaves (Zhao, Huang, & Keller, 2018). In maize leaves, Kocide treatment induced accumulation of glycolysis and TCA intermediates thereby enhancing energy metabolism and enhance branched chain amino acids accumulation, which play important role in oxidative phosphorylation energy source. Kocide resulted in increased levels of polyphenols such as 4-hydroxycinnamic acid, 1,2,4-benezenetiol, and their amino acid precursors (Phe and Tyr), thereby activating shikimate phenylpropanoid pathway in maize leaves suggesting induction of antioxidative processes. In cucumber plants, Kocide significantly affected the Arg/Pro pathway intermediates (Glu, Pro, Asp, Gln, Cit, putrescine, and fumarate). In both plants, Kocide exposure resulted in reduced levels of unsaturated fatty acids (linoleic and linolenic acids) and increased saturated fatty acid (laurate) indicating an alteration of plasma membrane homeostasis.

2.2.2. Silver based-NPs

Metabolomic studies suggest that the toxicity effects in plants exposed to *n*Ag is primarily attributed to the released Ag^+ ions (Chavez Soria et al., 2017; Zhang et al., 2018). In 7-day old *Arabidopsis thaliana* plants, a 24 h-exposure to 1 mg/L of citrate-*n*Ag, polyvinylpyrrolidone (PVP)-*n*Ag and $AgNO_3$ via roots resulted in alteration in six, three, and nine metabolites respectively, which were identified as fatty acid derivates. Targeted analysis of lipids showed that sphingolipids were significantly upregulated in Ag treatments, indicating disturbance in the vacuolar and plasma membrane of *A. thaliana* plants (Chavez Soria et al., 2017). In 4-week old cucumber plants, exposed to 10 and 100 mg/l *n*Ag, three times daily for consecutive 7-days via foliar spray, the responses were similar to ionic Ag exposure (Zhang et al., 2018). Both AgNPs and Ag^+ ion exposures altered the accumulation of 76 metabolites involved in activation of carbohydrate metabolism (TCA intermediates), antioxidant system and defense response (phenolic compounds, fatty acids). Ag treatments also resulted in decreased accumulation of Gln and Asn, responsible for N fixation, and over-accumulation of salicylic acid, which is a major signaling molecule that activates plant defense and systemic acquired resistance. Exposure to 100 mg/l *n*Ag enhanced the accumulation of linolenic and linoleic acids, the most abundant unsaturated fatty acids in the membrane (Zhang et al., 2018).

2.2.3. Other metal-based NPs

Maize plants exposed to soil amended with 100 mg/kg $n Fe_3O_4$, $n TiO_2$, or $n SiO_2$ for 28 days affected biological pathways related to defense

mechanisms and carbon metabolism (Zhao, Zhang, White, et al., 2019). $n$$Fe_3O_4$ induced maximum stress in maize tissues, followed by $n$$TiO_2$ and $n$$SiO_2$, expressed by increased accumulation of Tyr and Phe in leaves, which are precursor to phenylpropanoids, alkaloids and glucosinolates indicating activated defense response. They also accumulated polyamines (putrescine and spermine), GABA and its metabolites (Glu and succinate semialdehyde, respectively), which play a predominant role in stress response, nitrogen storage, signal transduction, growth and development. In maize leaves, TCA cycle intermediates, citric acid and a-ketoglutaric acid were increased whereas, succinic acid was decreased, affecting the carbon metabolism. The NPs altered several biological pathways in the leaves including amino acid metabolism (Arg/Pro, Gly/Ser/Thr), methane metabolism, pantothenate and CoA biosynthesis, carbon metabolism (TCA cycle, glycolysis, gluconeogenesis, pyruvate metabolism) and pyridine metabolism. In addition, $n$$Fe_3O_4$ and $n$$TiO_2$ specifically altered isoquinoline alkaloid biosynthesis and glyoxylate and dicarboxylate metabolism. In roots, all the ENMs affected inositol phosphate metabolism and TCA cycle, however, $n$$Fe_3O_4$ and $n$$TiO_2$ affected ascorbate/aldarate, methane, glycerolipid, glyoxylate, dicarboxylate metabolisms. $n$$TiO_2$ exposure (100–500 mg/l) to 7-day old rice (*Oryza sativa*) seedlings in hydroponic media for 14-days resulted in elevated levels of metabolites in TCA cycle and pentose phosphate pathway (glucose-6-phosphate, glucose-1-phosphate, succinic and isocitric acid), whereas decreased levels of metabolites (sucrose, isomaltulose, and glyoxylic acid) involved in the carbohydrate synthesis metabolism including starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism (Wu et al., 2017). Direct exposure to $n$$TiO_2$ suspension also increased the biosynthesis of fatty acids, amino acids and secondary metabolites in the rice (*Oryza sativa*) seedlings. In a recent study, Gong et al. reported that a 6-day exposure to 10 and 500 mg/l $n$$Y_2O_3$ disturbed carbohydrate metabolism, TCA cycle and amino acid metabolism in maize seeds (Gong et al., 2019). An increased accumulation of sugars (sucrose, fructose), free amino acids (Phe, Ty, Val, Ile, Leu, Ala, Thr, Asp, Asn, Glu, Gln, Pro), GABA, and organic acids (formate, malate, citrate, succinate, pyruvate) was attributed to cellular response to $n$$Y_2O_3$ stress.

Three weeks-old *Phaseolus vulgaris* plants were chronically exposed to 250 and 1000 mg/l $n$$CeO_2$ in the form of suspension every 48 h for 2 weeks via foliar spray or soil application, resulting in a total exposure load of 50 and 200 mg $n$$CeO_2$ (Salehi et al., 2018). Foliar application was shown to exhibit stronger negative effects; membrane lipids, lignans (sesaminol-2-O-β-glucoside and diphyllin), and metabolites involved in pteridin and lignin synthesis were enriched, demonstrating immediate response on structural integrity, whereas most carotenoids, glucosinolates, terpenoids, and

phenolic compounds were down-accumulated. In contrast, soil application resulted in overaccumulation of carotenoid and phenolic compounds, whereas polyols (ribitol, arabitol and pentitol) and pteridin-related compounds were down-accumulated. A three generational chronic exposure to nCeO$_2$ (500 mg/kg soil) exhibited higher degree of alterations in the metabolite accumulation in wheat (*Triticum aestivum*) grains compared to two-generational exposure (Rico et al., 2020). The altered metabolites were primarily involved in fructose-mannose metabolism, pentose-phosphate, phosphoribosyl pyrophosphate pathways; however, the degree of alteration in the grain metabolites was influenced by soil N availability.

Večeřová et al. (2016) separated the polar and non-polar fractions of the leaf extracts obtained from barley (*Hordeum vulgare*) plants exposed to an aerosol spray of CdO NPs (7–60 nm) for 21 days (Večeřová et al., 2016). LC-MS was used to determine sugars, phenolics, amino acids and Krebs cycle acids, while fatty acids were analyzed using GC-MS. An increase in the total amino acid content (especially Phe, Trp, Val, Leu, Asn, Tyr) in roots and leaves, indicated protective mechanisms in response to stress. Decrease in levels of sugars (pentose, hexose, disaccharides, 2-deoxy-D-ribose) in the roots, and phenolic acids (gallic acid, sinapic acid, ferulic acid) in leaves were reported. In a recent study, we performed targeted metabolomics in soybeans (*Glycine max*) exposed to CdS-quantum dots (CdS-QD) for 14 days in vermiculite soil media, which showed that major metabolic pathways in soybeans including glutathione metabolism, TCA cycle, glycolysis, fatty acid oxidation and biosynthesis were perturbed (Majumdar et al., 2019).

2.2.4. Carbon -based ENMs

In 7-day old *A. thaliana* plants grown on artificial media spiked with 125 and 1000 mg/l carbon nanodots (C-dots) for 7 days, growth was significantly inhibited compared to control and activated-carbon treatments. The metabolite profiling suggested increased accumulation of carbohydrates (melezitose, ribulose-5-phosphate, d-turanose, talose, d-glucopyranoside, inositol) in *A. thaliana* roots and shoots, which indicates impact on the carbohydrate metabolism and cell wall stress (Chen et al., 2018). In the C-dot exposed *A. thaliana* tissues, overaccumulation of various alkaloids, lignan, carotenoids, flavonoids, amino acids (Gly, Val, and homoserine (Hse)), organic acids (ethenedecanoic acids, 2-ketoadipic acid, hexacosinic acid, terephthalic acid, butyric acid, carboxylic acid), and fatty acids (heptadecanoic acid and octadecatrienoic acid) were noted, indicating activation of defense response. A three week chronic exposure of C60 fullerols via foliar spray twice a day for two days (1 mg and 2 mg/plant) to cucumber plants showed degradation of plasma membrane metabolites like linolenic

and palmitic acid, whereas increased accumulation of antioxidant metabolites such as 3-hydroxyflavone, 4-vinylphenol dimer, 1,4 benzenetriol, methyl transcinnamate, quinic acid, and dehydroascorbic acid (Zhao, Zhang, Wang, et al., 2019).

2.2.5. Root exudate metabolomics

Plant rhizosphere is rich in metabolites that exude from roots and assist plants to cope with abiotic or biotic stress by building tolerance, defense and resilience (Canarini et al., 2019). Zhao et al. reported that *n*Cu (10 and 20 mg/l) exposure triggered an increase in the release of amino acid from *C. sativus* plants to sequester Cu ions at ENM-root interface and decreased citric acid content to control Cu dissolution from *n*Cu, in order to lower Cu bioavailability and subsequent implications (Zhao, Huang, Hu, et al., 2016). Enhanced levels of ascorbic acid and phenolic compounds (benzoic acids, hydroxyvaleric acid, pelargonic acid, salicylic acid, and lactic acid) in the root exudate confirms increased stress in the plants exposed to *n*Cu (Zhao, Huang, Hu, et al., 2016). In another study, ENM (100 mg/kg nFe_3O_4, $nTiO_2$ and $nSiO_2$)-treated soil collected after 28 days plant growth showed decreased levels of water-soluble Cu and increased levels of levoglucosan, linolenic acid, 4-hydroxycinnamic acid, allo-inositol, β-mannosylglycerate, gluconic acid, methyl phosphate, and methyl β-D galactopyranoside compared to untreated soil, indicating exudation of compounds involved in defense response (Zhao, Zhang, White, et al., 2019). However, since the soil is a concomitant mixture of metabolites from the plant, residual organic matter and the soil microbiome, conclusions on root exudate characterization based on soil metabolomics could be confusing. Thus, sampling is crucial to extract maximum information from root exudate metabolomic studies, which may otherwise lead to inconclusive information. Appropriate control samples must be introduced while comparing ENM effects on root exudates between different plant species or varieties, as secondary metabolite composition vary significantly.

3. Proteomics: link between transcriptomics and metabolomics

Plant genome encodes for 36,795 proteins and the average size of a plant protein is 436 amino acid residues, which is 27% smaller than the average animal protein (Ramírez-Sánchez et al., 2016). Proteins are the key players in plant signaling and stress response as they are directly utilized in processes involved in cellular homeostasis. Characterizing the plant proteome offers functional analysis of the translated regions of the genome; hence, proteomics complements transcriptomic and metabolomic for a comprehensive understanding of underlying cellular mechanism in plants.

Proteomic analysis serves as an important tool in crop improvement, protection and phytotoxicity studies as it has the potential to identify the key regulatory proteins, posttranslation modifications and protein-protein interactions in plants in response to an external stimulus. Probing the ENM-plant interaction at the protein level is necessary to elucidate the cellular processes and identify the candidate proteins involved in ENM response. Using time-resolved experimental designs and by delving at subcellular or organelle levels, it is also feasible to identify ENM-responsive proteins involved in specific biological pathways at different growth stages of a plant (Komatsu et al., 2013). A rather unexplored area is the characterization of protein coronas formed around ENMs while interacting with plant tissues under different growth conditions, which also influence the toxicity and mobility of ENMs in plants.

3.1. Analytical techniques and challenges in plant proteomics

Two-dimensional gel electrophoresis (2DE) separates protein mixtures based on charge (isoelectric point) in the first dimension and by mass in the second dimension on 2D-gels, which in combination with MS have been used for decades for plant proteome profiling. However, it has its limitations owing to poor sensitivity, low reproducibility, and low throughput. Especially for plant whole lysates that contain an array of secondary metabolites like pigments and phenolics, these compounds can introduce streaking in a gel (Vannini et al., 2014). With rapid development in MS techniques, quantitative proteomics have evolved from gel-based to gel-free approaches. Like metabolomics, proteomics can also be categorized into untargeted and targeted method. Due to the exploratory nature of untargeted proteomics, it is the preferred approach to screen for candidate protein markers to elucidate plant responses to ENM exposure. This approach can facilitate high-throughput analysis of protein abundance across different ENM exposures, tissue types, stages of growth, physiological conditions and stress conditions (Hart-Smith et al., 2017). In a gel-based approach, proteins are separated by 2DE and the spots showing comparable differences are excised from the gel. The proteins in the gel fractions are digested into peptides, which are then characterized by LC-MS. A typical gel- and label-free quantitative proteomic analysis used for discovery studies employ a bottom-up approach, where the proteins in a sample are cleaved into peptides, which are then separated, identified and quantified using LC-MS/MS (Figure 3) (Hu et al., 2015). The steps and key challenges in proteomic analysis in ENM-plant interaction study are summarized below.

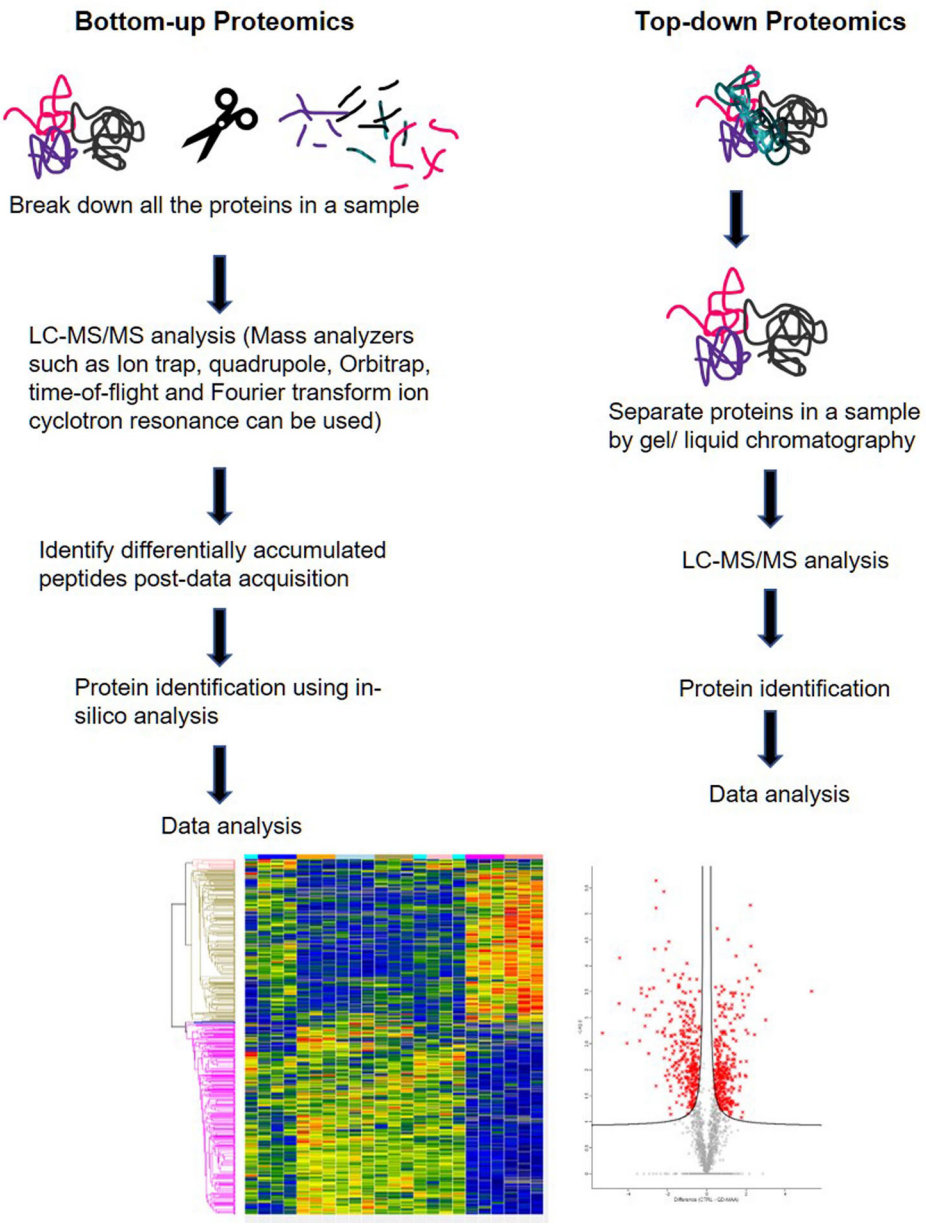


Figure 3. Proteomic analysis in plants exposed to ENMs.

3.1.1. Sampling, quenching and extraction

Proteomic analysis demands robust sampling methods aligned with the scope of the study. Combining different organs (root/leaf/fruits) into one sample must be avoided as the tissue-specific protein information is lost, resulting in confounding interpretation of results. In addition, proteomics result is also prone to variation in response to age, time of harvest, and growth conditions. Hence, control experiments and biological replicates

should always be carried out simultaneously and harvested at the same time of the day. Plant tissues must be immediately frozen upon harvest, followed by homogenization into fine powder which can be stored at −80 °C. The proteins in the sample are then subjected to precipitation, resuspension in buffer, followed by digestion using protease trypsin, which are then analyzed using LC-MS. The major challenges in sample preparation for proteomics arise from recalcitrant tissues, hydrophobic membrane proteins, presence of lignin, proteases, and metabolites like phenolic compounds, lipids, organic acids, carbohydrates, terpenes, and pigments, and presence of high abundance proteins that may mask variation in low abundance proteins during analysis (Wang et al., 2008).

3.1.2. Data acquisition

Electrospray ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI) are the most common platforms used to ionize peptides and proteins. The precise molecular mass of the resulting ions is then analyzed using mass analyzers, such as ion trap, quadrupole, Orbitrap, time-of-flight (TOF), and Fourier transform ion cyclotron resonance (FTICR), which are often used in tandem to achieve higher degrees of ion separation (Lai et al., 2012). In untargeted proteomics using LC-MS/MS, data dependent acquisition is performed, where the highest abundance peptide ions from full MS scans are selected for MS/MS. However, this may generate datasets skewed toward the identification of relatively high abundance proteins, thereby masking and excluding the low abundance proteins from quantification (Hart-Smith et al., 2017). Several labeling techniques such as isobaric Tags for Relative and Absolute Quantitation (iTRAQ) and Stable Isotope Labeling by Amino acids in Cell culture (SILAC) are also available which can help to reduce errors introduced during measurement conditions. However, untargeted proteomic analysis requires extensive data processing and is currently challenged by incomplete and limited nature of plant genomic and proteomic databases. In recent times, the use of label-free shotgun proteomic techniques have become increasingly popular, as they do not restrict the number of proteins identified compared to gel-based methods (Majumdar et al., 2015, 2019; Mirzajani et al., 2014; Vannini et al., 2014; Verano-Braga et al., 2014). However, gel-free methods have several drawbacks, such as masking of low abundant peptides and unavailability of protein database for all species.

3.1.3. Data processing

Untargeted proteomics require extensive postacquisition data processing after extracting features using LC-MS. There are several open-access and

commercial software tools that can be used for feature extraction, such as MaxQuant, Proteome Discover, OpenMS, etc. The extracted features are subjected to filtering, peak detection, normalization, spectral identification, retention time/peak alignment, statistical analysis, peptide identification, quantitation, protein inference, and data visualization. All the raw and processed proteomics data should be deposited into public data repositories.

Targeted proteomics, on the other hand, is hypothesis-driven and provides unparallel specificity and sensitivity for the targeted proteins of biological interest. In targeted proteomics, the mass spectrometer is programed to analyze a preselected group of proteins, using SRM, whereby assays are developed on a QqQ to detect fragment-ion signals arising from unique peptides representing each targeted protein. The most common software platform used for processing targeted proteomics data is Skyline (MacLean et al., 2010).

3.2. Application of proteomics to probe interactions at ENM-plant interface

Comparative assessment of plant proteomes in response to ENMs can effectively screen candidate proteins and associated pathways, and potentially elucidate the cross talk between different processes that control the metabolite regulation in the cells. Several studies have employed untargeted proteomics to identify key plant proteins involved in ENMs-associated cellular signaling or stress response. We discuss nine *in vivo* studies that have used proteomics to explore the alterations in plants at the protein level, in response to metal-based NPs, including *n*Ag, $n CeO_2$, *n*Al, $n Al_2O_3$, *n*Fe, *n*ZnO and CdS QDs (Table 2).

Impact of bare or surface-functionalized *n*Ag on plant proteomes have been studied in several plants, including arugula, wheat, rice, soybeans, and tobacco. In rice plants exposed to 30 and 60 mg/l *n*Ag for 20 days in hydroponic growth media, 2DE-nanoLC/FTICR MS identified twenty-eight differentially-accumulated proteins, which were primarily involved in oxidative stress response, Ca^{2+} regulation and signaling, transcription, and protein synthesis/degradation, cell wall damage, and apoptosis (Mirzajani et al., 2014). *n*Ag exposure incremented levels of defense-related proteins including SOD, glutathione-S-transferase (GST), L-ascorbate peroxidase, which has been shown to result from Ag^{+} leaching. Similar defense response expressed by elevated levels of SOD and GST was reported in arugula (*Eruca sativa*) and wheat plants, respectively, exposed to 10 mg/l polyvinylpyrrolidone (PVP)-coated *n*Ag for 5 days (Vannini et al., 2014). Comparative proteomic response in the roots of arugula plants exposed to *n*Ag and $AgNO_3$ suggested that both treatments disrupt redox regulation, biosynthesis of sulfur containing amino acids, and cellular homeostasis.

Table 2. Summary of proteomic studies in plants exposed to ENMs.

ENM	Size	Plant species	Age at exposure (days)	Growth media	Exposure route	Exposure period	Exposure concentration	Tissue analyzed	Instrument used	Pathways associated with altered proteins	Reference
*n*Ag	18 nm	*Oryza sativa*	10	Artificial media	Root	20 days	30,60 mg/l	Whole plant	2-DE, nanoLC-LTQ/ FT-ICR MS	Oxidative stress, Ca^{2+} regulation and signaling, Transcription, Protein degradation, Cell wall damage, Apoptosis	(Mirzajani et al., 2014)
*n*Ag (PVP-coated)	10 nm	*Eruca sativa* Mill.	0	Filter paper	Seeds	Soaked in suspension for 4 h followed by 5 days on filter paper	10 mg/l	Root	2DE, nanoLC-Q-TOF MS	Sulfur metabolism, Cysteine biosynthesis, Methionine biosynthesis, Oxidative stress response	(Vannini et al., 2013)
*n*Ag (PVP-coated)	10 nm	*Triticum aestivum* L. cv Blasco	0	Filter paper	Seeds	Soaked in suspension for 4 h followed by 5 days on filter paper	10 mg/l	Root, shoot	2DE, nanoLC-Q-TOF MS	Photosystem biogenesis, Electron transport chain, Energy metabolism, Cell wall biosynthesis, Sulfur Amino acid biosynthesis, Oxidative stress response	(Vannini et al., 2014)
*n*Ag (citrate-coated)	50nm	*Nicotiana tabacum* var. Burley	60	Milli-Q water	Root	7 days		Root, Leaf	2DE, MALDI-TOF/TOF MS	*Root* Defense and oxidative stress response *Leaf* Carbohydrate metabolism, Energy metabolism, Photosynthesis, Electron transport chain	(Peharec Štefanić et al., 2019)
*n*Ag	15 nm	*Glycine max*	7	De-ionized water	Root	3 days	50 ppm	Root, leaf	nanoLC/LTQ XL Orbitrap	*Root* Secondary metabolism, Cell organization, Hormone metabolism, Lipid metabolism, and Protein synthesis *Leaf* Calvin cycle, tricarboxylic acid cycle, Lipid metabolism, Carbohydrate metabolism, Protein degradation, Tetrapyrrole synthesis, Amino acid metabolism	(Hossain et al., 2016)
nAl_2O_3	30–60 nm	*Glycine max*	7	De-ionized water	Root	3 days	500 ppm	Root, leaf	nanoLC/LTQ XL Orbitrap		(Hossain et al., 2016)
*n*ZnO	< 50 nm	*Glycine max*	7	De-ionized water	Root	3 days	500 ppm	Root, leaf	nanoLC/LTQ XL Orbitrap		(Hossain et al., 2016)
nCeO_2	67 × 8 nm	*Phaseolus vulgaris* L. (var. Red hawk)	0	Soil	Root	96-102 days	62.5-500 mg/kg	Fruit (seed)	nano-LC/Q-Exactive MS	Storage, Carbohydrate metabolism, Protein folding, Defense response, Fe binding	(Majumdar et al., 2015)

(*continued*)

Table 2. Continued.

ENM	Size	Plant species	Age at exposure (days)	Growth media	Exposure route	Exposure period	Exposure concentration	Tissue analyzed	Instrument used	Pathways associated with altered proteins	Reference
$nCeO_2$	10–30 nm	*Phaseolus vulgaris* L. (var. pinto bean)	21	Soil	Root	14 days (48h chronic application)	250-2000 mg/l	Leaf	nano-LC/hybrid QTOF-MS	Carbon fixation, Photosynthesis, Protein biosynthesis and turnover, Oxidative stress	(Salehi et al., 2018)
					Foliar	15 days (48h chronic application)	250-2000 mg/l	Leaf	nano-LC/hybrid QTOF-MS	Photosynthesis, Electron transport chain, Protein biosynthesis and turnover, Oxidative stress, Fatty acid biosynthesis, Fe binding	(Salehi et al., 2018)
CdS Quantum dots	8 nm	*Glycine max*	11	Vermiculite media	Root	14 days	200 mg/l	Root	nano-LC/Q-Exactive MS	Glycolysis, TCA cycle, Urate oxidation, ATP synthesis-coupled-proton transport, β-oxidation of fatty acids, Jasmonic acid and sphingosine biosynthesis, Lignin biosynthesis, Defense response, Ion binding, Channel activity, Membrane organization, Ca^{2+}-transporting ATPase activity, Pentose phosphate pathway, Glucuronate pathway, Calvin cycle, Glycolysis/ gluconeogenesis, Amino acid biosynthesis, Catecholamine biosynthesis, GABA shunt, Phenylpropanoid pathway, GSH metabolism, Isoflavonoid synthesis, Carbon fixation, Glyoxylate/dicarboxylate metabolism, Terpenoid biosynthesis, Sucrose and starch catabolism	(Majumdar et al., 2019)
$KAuCl_4$ (Biotransformation to *n*Au)	NR	*Arabidopsis thaliana* (Col- 0)	5	Artificial media	Root	5 days	10 ppm of $KAuCl_4$	Root, shoot	2-DE, MALDI-TOF-MS	*Root* Oxidative stress response *Shoot* Pentose phosphate shunt, Oxidative stress response, Carbohydrate metabolism, Electron transport chain	(Tiwari et al., 2016)

However, *n*Ag also alter endoplasmic reticulum and vacuole-associated proteins in arugula and wheat roots, not demonstrated in the Ag^{+} treatments. Vannini et al. also reported an increased production of malate dehydrogenase in roots, which reportedly increased root exudation of organic acids such as citrate, oxalate, malate, succinate and acetate (Vannini et al., 2014). Zhao et al. demonstrated in a metabolomic study that uptake and translocation of *n*Cu in cucumber plant triggers a feedback control mechanism in tandem to modulate the root exudation of amino acids and organic acids for defense response and restricting ion release, to maintain cellular homeostasis (Zhao, Huang, Hu, et al., 2016). In tobacco (*Nicotiana tabacum*) plants, a 7-day exposure to citrate-coated *n*Ag in tobacco plants altered the proteins related to defense response and oxidative stress, at a similar level as $AgNO_3$ treatments; however, although both *n*Ag and $AgNO_3$ affected mostly photosynthesis related proteins, in the leaves the effect was significantly higher in *n*Ag exposed plants, highlighting a nano-specific response (Peharec Štefanić et al., 2019). In another study, proteomic analysis in soybean plants after 3-days of root exposure to three different metal (-oxide) nanoparticles showed significantly negative response to 5 ppm *n*Ag treatments compared to 500 ppm $n Al_2O_3$ and 500 ppm *n*ZnO (Hossain et al., 2016). The drastic decrease in the proteins related to energy metabolism and a compromised defense system in the *n*Ag exposed plants thus resulted in decreased growth of soybean plants, compared to the control, $n Al_2O_3$ and *n*ZnO exposures.

Proteomic analysis of soybean roots exposed to 200 mg/l of differentially coated-CdS-QDs (uncoated, mercaptoacetic acid, polyvinylpyrrolidone, trioctylphosphine oxide or glycine-coated) in vermiculite showed over-accumulation and under-accumulation of 99 and 44 root proteins, respectively, irrespective of coating type (Majumdar et al., 2019). The response was also compared to bulk-equivalent and Cd^{2+} ion treatments. The affected proteins unique to QD exposures were involved in glycolysis, TCA cycle, urate oxidation, and ATP synthesis-coupled-proton transport. Stress signaling pathways were also upregulated, especially β-oxidation of fatty acids, biosynthesis of jasmonic acid and sphingosine, and lignin biosynthesis. Some proteins involved in defense response, ion binding, channel activity, and membrane organization were negatively affected. Ca^{2+}-transporting ATPase activity was also downregulated in all CdS-QD-treated roots. reported in *n*Ag treatments as well. Some altered proteins that were common to CdS bulk particles and Cd^{2+} ion exposure were those associated with pentose phosphate pathway, glucuronate pathway, Calvin and TCA cycle, glycolysis/gluconeogenesis, amino acid biosynthesis, catecholamine biosynthesis, GABA shunt, phenylpropanoid pathway, GSH metabolism, isoflavonoid synthesis, carbon fixation, glyoxylate/dicarboxylate metabolism, jasmonic acid biosynthesis, and terpenoid biosynthesis, and sucrose and starch catabolism.

Although exploration of the plant proteome can deliver a wealth of information, the studies concerning ENMs have been primarily focused on toxicity. Tiwari et al. (2016) employed gel-based proteomics and transcriptomics to elucidate the mechanism of biotransformation of $KAuCl_4$ to *n*Au in 5-day old *A. thaliana* plants (Tiwari et al., 2016). A total of 10 and 15 spots from 2DE of root and shoot samples, respectively, were digested into peptides by trypsin, and analyzed using MADI-TOF-MS. *n*Au affected carbohydrate metabolism, electron transport chain and oxidative stress in plant tissues. The production of GSTs in *A. thaliana* shoots in response to increasing Au accumulation, suggested they play an important role in controlling oxidative stress during the reduction of Au ions to *n*AuNPs. A 14-day exposure to 250–1000 mg/l $nCeO_2$ via foliar spray and root absorption resulted in significant effect on carbon fixation and energy production in pinto bean (*Phaseolus vulgaris* L., var. pinto) plants (Salehi et al., 2018). This involved enhanced production of thylakoid proteins (light-dependent) participating in photosynthesis, decreased production of RuBisCo and altered enzyme activities in the electron transport chain in mitochondria $nCeO_2$ have shown to be very actively involved in the oxidative chemistry in plant cells, either acting as an antioxidant enzyme mimic or a stress elicitor (Nelson et al., 2016; Wu et al., 2017). In pinto beans, two key enzymes responsive to oxidative stress, ascorbate peroxidase and glutathione peroxidase were down-accumulated. Altered response of ascorbate peroxidase enzymes has also been reported at biochemical level in kidney beans (*P. vulgaris L.,* var. Red Hawk) (Majumdar et al., 2015) and tomato (*Solanum lycopersicum*) (Barrios et al., 2016), as well as at transcriptomics level in *A. thaliana* (Tumburu et al., 2015). Interestingly, transcription factors (Elongation factor 1-alpha), which play a central role in protein biosynthesis and turnover were shown to be downregulated in the leaves (Salehi et al., 2018) as well as first generation seeds of bean plants exposed to 125–500 mg/kg $nCeO_2$ (Majumdar et al., 2015). Lipoxygenase, a protein responsible for fatty acid biosynthesis, iron binding, and oxido-reductase activity was also down-accumulated in bean leaves and seeds (Majumdar et al., 2015; Salehi et al., 2018). The differentially-regulated proteins in the seeds obtained from $nCeO_2$ treated parent plants were mostly down-accumulated compared to those harvested from untreated controls, and a dose-dependent increase in the number of candidates were noted. The candidate proteins were involved primarily in storage (phaseolin, legumin), carbohydrate metabolism (lectins), protein folding, and resistance mechanism (Majumdar et al., 2015).

4. Other omics domains applied in nano-enabled agriculture

Recent advances in tools for genomics and transcriptomics in conjunction with metabolomics and proteomics have the potential to accelerate

agricultural development (Gómez-Merino et al., 2015). An area that can benefit substantially from these approaches is the plant-microbe interaction. Microbial communities play an important role in plant growth and productivity by directly controlling soil processes like stabilizing soil structure, nutrient bioavailability, degradation of organic pollutants, CO_2 fixation and C degradation. They are however highly sensitive and susceptible to toxicity from stressors. Next generation sequencing technologies such as pyrosequencing and Illumina-based sequencing has resolved complexities in the microbial community with higher accuracy than conventional methods (Xu & Wang, 2019). Metagenomics allows to collectively characterize genome sequences of known and unknown members of the entire microbial community in an environmental sample, without having to isolate each into pure cultures. ENMs present in the agricultural soil from intentional use of nano-enabled agrochemicals or unintentional incorporation in the biosolids or irrigation water have the potential to impact the soil microbial community thereby affecting the agricultural productivity (Judy et al., 2015). Metagenomic analysis of the soil microbial community provide potential means to design sustainable ENMs with potential to bolster plant protection against pests and enhance productivity and nutritional quality (Judy et al., 2015; Metch et al., 2018). However, although the metagenomic analysis provide important information on the functional capacity of the soil microbial community, it does not reflect the metabolic activities of individual species or the community.

Genomic interpretation can be complemented with gene expression analysis at the RNA level, also known as transcriptomics, which can provide critical information on sustainable ENM design for agricultural applications and impact on plants and soil microbial community. Advances in gene expression analysis using RNA-seq analysis have evolved the understanding of alteration in gene expression in complex samples, which can be further validated by RT-PCR analysis of targeted genes. The transcriptomic studies in plants exposed to ENMs have been very well reviewed in two reviews, where the authors discussed the integration of transcriptomics, micro-RNA and proteomics data in plants for discovering nano-specific biomarkers and effects (Ruotolo et al., 2018; Singh et al., 2017). For transcriptomic studies, the knowledge of the whole genome sequence and their functional annotation is critical and hence is better addressed in smaller genomes. *A. thaliana* has the smallest genome (145 Mbp) that is completely annotated unlike the crop species like rice (450 Mbp) or soybeans (1115 Mbp), and hence all global transcriptome studies focusing on ENM-plant interactions have been carried out in *A. thaliana* to avoid complexity (Ruotolo et al., 2018). However, complementary proteomics and metabolomics can be used to functionally annotate the genes of interest in non-model species. In their

review, Ruotolo et al. concluded that the plants induce defense mechanisms against ENM exposure which are resolved at the transcriptome and proteome level and are primarily related to modification of root architecture, phytohormone signaling and antioxidant system activation (Ruotolo et al., 2018).

5. Integration of multiomics

Integration of multiomics in plants provides a comprehensive knowledge on the regulatory mechanism at multiple subcellular organization levels in response to an external stimulus. Although the number of plant studies employing individual omic techniques to identify key biomolecules in response to ENMs is increasing, only a few studies have integrated different omics (Chen et al., 2018; Majumdar et al., 2019; Salehi et al., 2018; Zhao, Zhang, Wang, et al., 2019). It is critical to integrate the response at all levels, including metabolome, proteome and transcriptome to identify the molecular underpinnings that regulate the metabolic pathways in response to ENMs, which eventually is expressed in the phenotype. However, there are multiple challenges that have slowed down the use of integrative approaches, especially in crop species. The first challenge is the unavailability of transcriptomic databases and incomplete or un-annotated proteomic databases for non-model species. The second bottleneck is the difficulty in scaling very large datasets from different levels within the phenome. Being closest to the phenotype, the metabolome is easily influenced by immediate environmental conditions, which may or may not correlate with genomic and transcriptomic profile. Hence, the emergent properties at the higher-level organization of the plant (phenotype, metabolome, or proteome) are not fully determined by the properties of the lower levels (transcriptome or genome) (do Amaral & Souza, 2017). Thirdly, metabolomic analysis is often oversimplified due to the common assumption that the sampling is performed in metabolic steady-state, characterized by constant levels of metabolites and that different metabolic pathways operate in isolation. At the whole plant level of integration, there are too many complex and dynamic processes occurring simultaneously which regulate feedback mechanisms in the cellular metabolism.

In order to integrate the data from different omics in a test specimen, it is necessary to use the same sample for aliquoting into fractions for individual omic analysis. Assuming that the data acquired from each omic is of high quality and validated, they can be integrated by different approach, including (1) postanalysis data integration; (2) integrated data analysis; and (3) systems modeling methods (Pinu et al., 2019). In postanalysis data integration, the omic datasets are analyzed in isolation and the key features are networked in an overall model pathway. In integrated data analysis,

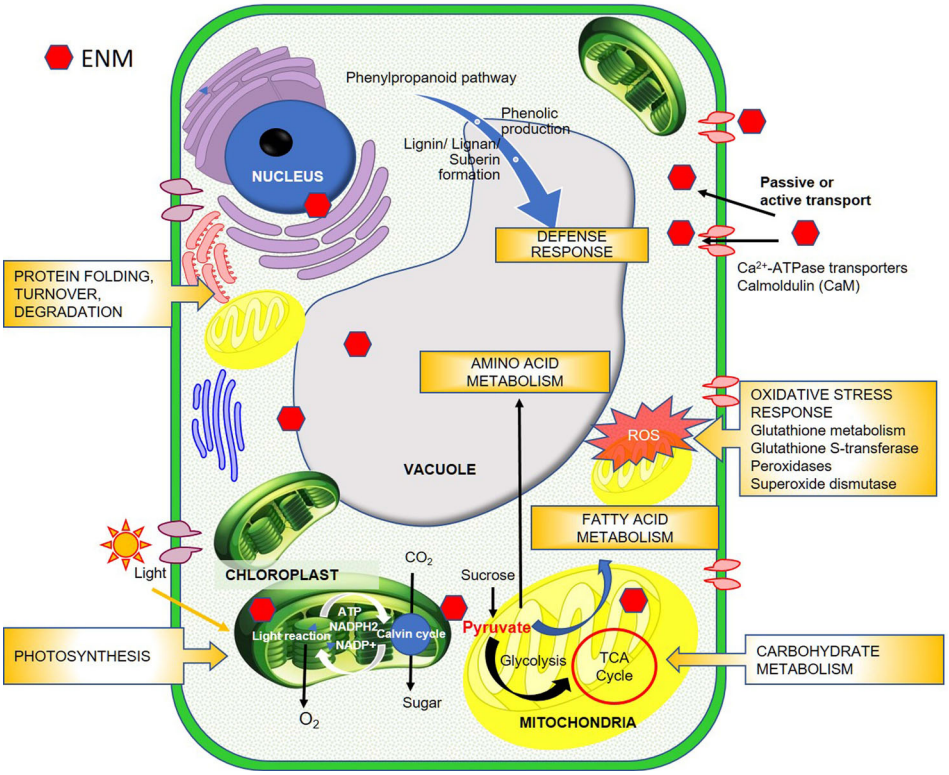


Figure 4. Integration of omics to elucidate ENM responsive biological pathways.

specialized tools are used to merge different omics data sets prior to data analysis and interpretation. Systems modeling approaches incorporate modeling tools utilizing preexisting comprehensive omic databases (Pinu et al., 2019). Only a handful of studies have utilized integrative approach in ENM-plant interaction studies and have relied primarily on postanalysis data integration (e.g. Chen et al., 2018; Majumdar et al., 2019; Salehi et al., 2018; Zhao, Zhang, Wang, et al., 2019).

Integration of the plant metabolome with the proteome and transcriptome can answer several questions regarding ENM-associated mechanisms, including routes of entry, translocation, defense response and toxicity (Figure 4). Individual omic studies across different plant species and postanalysis integration studies over the past seven years have highlighted a few metabolic pathways of interest, depending on the mode of exposure. In root exposure studies, ENMs have shown to induce oxidative stress in the root tissues due to the immediate availability of metal ions from ENM dissolution, which is appreciated by the release of acidic exudates (Majumdar et al., 2019; Mirzajani et al., 2014; Peharec Štefanić et al., 2019; Zhao, Huang, Hu, et al., 2016). As a defense response to ENMs, multiple metabolic pathways have been reported to be differentially regulated, including

glutathione metabolism, GABA shunt, phenylpropanoid pathway, shikimate pathway, and flavonoid pathway. Different phenolic compounds and amino acids are evidently altered in these pathways in order to defend the plants from reactive oxygen species (ROS). Another common sign of stress in the plant roots is an increase in lignin content and alteration of membrane lipids, which protect the roots from additional stress (Majumdar et al., 2019). In several studies, *n*Cu and CdS-QDs exposure altered levels of Ca-binding proteins, such as calmodulin or Ca^{2+}-ATPase (Majumdar et al., 2019; Mirzajani et al., 2014). It is hypothesized that ENMs often bind to Ca^{2+} receptors or use the Ca-channels or Ca^{2+}-ATP pumps to enter the plants. This disturbs the Ca^{2+} flux in the plant cell, thereby resulting in ROS formation. The glutathione pathway and the GABA shunt play a major role in ROS defense in plants affected by exposure to ENMs. In addition to defense responses in different plant tissues, ENMs have been shown to influence carbon fixation, amino acid metabolism, and photosynthesis in the aerial tissues. In a recent study, metabolomics of leaf mesophyll protoplast showed that *n*Fe and Mn_3O_4 enhanced the photosynthetic quantum yield (Wang et al., 2020). However *n*MoS_2 and *n*Ag had negative effects (Wang et al., 2020). Carbohydrate metabolism including glycolysis and TCA cycle are the most sensitive pathways that can be easily altered by ENMs (Tables 1 and 2, Figure 4). The mechanism deduced by integrative omic studies could be utilized to design sustainable ENMs for desired applications. Identification of the transporter channels/proteins in the plants mediating the entry of ENMs, associated with the feedback metabolic processes in the leaf chloroplast and mitochondria, will allow efficient use of ENMs for targeted applications.

6. Concluding remarks and future perspectives

Sustainable advancement of the agricultural industry requires interdisciplinary and convergent approaches to meet growing demands and maximize resource optimization. Nanotechnology has demonstrated strong potential to promote growth and bolster resistance and resilience in crops against biotic and abiotic factors. In addition, nanotechnology also provides increased flexibility and sensitivity to be used as targeted delivery platforms and sensors to probe plant and environmental signals. However, an efficient application of nanotechnology in agriculture should harness the mechanistic understanding of the interactions at the subcellular level provided by omic techniques. This review summarizes the studies taking advantage of cutting-edge high-throughput omic approaches that can be utilized to address critical concerns in applications and implications of nanotechnology in agriculture. The advancements in analytical capabilities

as well as bioinformatic tools provides an opportunity to integrate information acquired at multiple organizational levels and address in-depth understanding of mechanisms influencing biological processes or responses. The integration of data from ENM and plant interaction studies is still in its nascent stage and needs more attention. These studies will enable development of biomarkers in plants for nanotoxicity or for potential application of ENMs such as increased crop productivity and systemic acquired resistance. A knowledge base developed from the integration of multiomic tools can also help in designing ENM based-sensors for detecting stress signaling molecules, which can then be programed to initiate controlled release of agrochemicals. Due to their high-throughput nature, multiomics can be used to study ENM-plant interactions at different growth stages and environmental conditions to find the optimum condition for an application.

Even though model plant species such as *A. thaliana* have served as the gold standard for molecular studies, with the advent of more accessible metabolomics, functional proteomics and genomics, there will be an increasing use of crop species with partially or fully sequenced genomes. Most omic studies currently use untargeted approaches to develop hypothesis and compare different conditions, including plant species, nanoparticle type and growth conditions, and to find biomarkers of exposure, stress and susceptibility. The next step will be to apply targeted approaches to validate the hypotheses and effectively quantify the key genes, proteins or metabolites that play a dominant role in negative or beneficial effects. With a recent growing number of omic studies in plants and the confounding variations associated with exposure conditions and ENM used, it is extremely important for the research community to realize the importance of reporting precise details on environmental conditions, plant age, ENM size and surface characteristics, ENM stability in the exposure media, exposure route, instrumental conditions used for omic analysis, data handling and analytical challenges experienced during the study. For a holistic and continuous progress in utilizing the immense potential of nanotechnology in agriculture and food industry, such experimental details are instrumental for attaining reproducibility, confidence and better utilization for practical applications.

Acknowledgements

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Funding

This material is based upon work supported by the National Science Foundation under Grant No. 1901515.

ORCID

Sanghamitra Majumdar http://orcid.org/0000-0002-8742-4759
Arturo A. Keller http://orcid.org/0000-0002-7638-662X

References

Avellan, A., Yun, J., Zhang, Y., Spielman-Sun, E., Unrine, J. M., Thieme, J., Li, J., Lombi, E., Bland, G., & Lowry, G. V. (2019). Nanoparticle size and coating chemistry control foliar uptake pathways, translocation, and leaf-to-rhizosphere transport in wheat. *ACS Nano*, *13*(5), 5291–5305. https://doi.org/10.1021/acsnano.8b09781

Barrios, A. C., Rico, C. M., Trujillo-Reyes, J., Medina-Velo, I. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2016). Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. *The* Science of the Total Environment, *563-564*, 956–964. https://doi.org/10.1016/j.scitotenv.2015.11.143

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, *10*(157), 157. https://doi.org/10.3389/fpls.2019.00157

Castillo-Michel, H. A., Larue, C., Pradas del Real, A. E., Cotte, M., & Sarret, G. (2017). Practical review on the use of synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption spectroscopy to investigate the interactions between plants and engineered nanomaterials. *Plant Physiology and Biochemistry: PPB*, *110*, 13–32. https://doi.org/10.1016/j.plaphy.2016.07.018

Chavez Soria, N. G., Montes, A., Bisson, M. A., Atilla-Gokcumen, G. E., & Aga, D. S. (2017). Mass spectrometry-based metabolomics to assess uptake of silver nanoparticles by *Arabidopsis thaliana*. *Environmental Science: Nano*, *4*(10), 1944–1953. https://doi.org/10.1039/C7EN00555E

Chen, J., Liu, B., Yang, Z., Qu, J., Xun, H., Dou, R., Gao, X., & Wang, L. (2018). Phenotypic, transcriptional, physiological and metabolic responses to carbon nanodots exposure in *Arabidopsis thaliana* (L.). *Environmental Science: Nano*, *5*(11), 2672–2685. https://doi.org/10.1039/C8EN00674A

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D. S., & Xia, J. (2018). MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research*, *46*(W1), W486–W494. https://doi.org/10.1093/nar/gky310

Conway, J. R., Beaulieu, A. L., Beaulieu, N. L., Mazer, S. J., & Keller, A. A. (2015). Environmental stresses increase photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. *ACS Nano*, *9*(12), 11737–11749. https://doi.org/10.1021/acsnano.5b03091

Cornelis, G., Hund-Rinke, K., Kuhlbusch, T., van den Brink, N., & Nickel, C. (2014). Fate and bioavailability of engineered nanoparticles in soils: A review. *Critical Reviews in Environmental Science and Technology*, *44*(24), 2720–2764. https://doi.org/10.1080/10643389.2013.829767

Dimkpa, C. O., Singh, U., Bindraban, P. S., Elmer, W. H., Gardea-Torresdey, J. L., & White, J. C. (2018). Exposure to Weathered and fresh nanoparticle and ionic zn in soil promotes grain yield and modulates nutrient acquisition in wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry*, *66*(37), 9645–9656. https://doi.org/10.1021/acs.jafc.8b03840

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature*, *411*(6839), 843–847. https://doi.org/10.1038/35081178

Djanaguiraman, M., Nair, R., Giraldo, J. P., & Prasad, P. V. V. (2018). Cerium oxide nanoparticles decrease drought-induced oxidative damage in sorghum leading to higher photosynthesis and grain yield. *ACS Omega*, *3*(10), 14406–14416. https://doi.org/10.1021/acsomega.8b01894

do Amaral, M. N., & Souza, G. M. (2017). The challenge to translate OMICS data to whole plant physiology: The context matters. *Frontiers in Plant Science*, *8*, 2146–2146. https://doi.org/10.3389/fpls.2017.02146

Du, W., Gardea-Torresdey, J. L., Ji, R., Yin, Y., Zhu, J., Peralta-Videa, J. R., & Guo, H. (2015). Physiological and biochemical changes imposed by CeO2 nanoparticles on wheat: A life cycle field study. *Environmental Science & Technology*, *49*(19), 11884–11893. https://doi.org/10.1021/acs.est.5b03055

Elmer, W., De La Torre-Roche, R., Pagano, L., Majumdar, S., Zuverza-Mena, N., Dimkpa, C., Gardea-Torresdey, J., & White, J. C. (2018). Effect of metalloid and metal oxide nanoparticles on fusarium wilt of watermelon. *Plant Disease*, *102*(7), 1394–1401. https://doi.org/10.1094/PDIS-10-17-1621-RE

Etalo, D. W., De Vos, R. C. H., Joosten, M. H. A. J., & Hall, R. D. (2015). Spatially resolved plant metabolomics: Some potentials and limitations of laser-ablation electrospray ionization mass spectrometry metabolite imaging. *Plant Physiology*, *169*(3), 1424–1435. https://doi.org/10.1104/pp.15.01176

FAO, IFAD, UNICEF, WFP, & WHO. (2019). *The state of food security and nutrition in the world 2019* (No. 978-92-5-130571-3). http://www.fao.org/3/ca5162en/ca5162en.pdf

Fiehn, O. (2002). Metabolomics–The link between genotypes and phenotypes. *Plant Molecular Biology*, *48*(1/2), 155–171. https://doi.org/10.1023/A:1013713905833

Fiehn, O., Sumner, L. W., Rhee, S. Y., Ward, J., Dickerson, J., Lange, B. M., Lane, G., Roessner, U., Last, R., & Nikolau, B. (2007). Minimum reporting standards for plant biology context information in metabolomic studies. *Metabolomics*, *3*(3), 195–201. https://doi.org/10.1007/s11306-007-0068-0

Garner, K. L., Suh, S., & Keller, A. A. (2017). Assessing the risk of engineered nanomaterials in the environment: Development and application of the nanofate model. *Environmental Science & Technology*, *51*(10), 5541–5551. https://doi.org/10.1021/acs.est.6b05279

Giese, B., Klaessig, F., Park, B., Kaegi, R., Steinfeldt, M., Wigger, H., von Gleich, A., & Gottschalk, F. (2018). Risks, Release and concentrations of engineered nanomaterial in the environment. *Scientific Reports*, *8*(1), 1565. https://doi.org/10.1038/s41598-018-19275-4

Giraldo, J. P., Wu, H., Newkirk, G. M., & Kruss, S. (2019). Nanobiotechnology approaches for engineering smart plant sensors. *Nature Nanotechnology*, *14*(6), 541–553. https://doi.org/10.1038/s41565-019-0470-6

Gómez-Merino, F. C., Trejo-Téllez, L. I., & Alarcón, A. (2015). Plant and microbe genomics and beyond: Potential for developing a novel molecular plant nutrition approach. *Acta Physiologiae Plantarum*, *37*(10), 208. https://doi.org/10.1007/s11738-015-1952-2

Gong, C., Wang, L., Li, X., Wang, H., Jiang, Y., & Wang, W. (2019). Responses of seed germination and shoot metabolic profiles of maize (*Zea mays* L.) to Y2O3 nanoparticle stress [10.1039/C9RA04672K. *RSC Advances*, *9*(47), 27720–27731. https://doi.org/10.1039/C9RA04672K

Guo, H., White, J. C., Wang, Z., & Xing, B. (2018). Nano-enabled fertilizers to control the release and use efficiency of nutrients. *Current Opinion in Environmental Science & Health*, *6*, 77–83. https://doi.org/10.1016/j.coesh.2018.07.009

Hart-Smith, G., Reis, R. S., Waterhouse, P. M., & Wilkins, M. R. (2017). Improved quantitative plant proteomics via the combination of targeted and untargeted data acquisition. *Frontiers in Plant Science*, *8*, 1669–1669. https://doi.org/10.3389/fpls.2017.01669

He, X., Deng, H., & Hwang, H-m. (2019). The current application of nanotechnology in food and agriculture. *Journal of Food and Drug Analysis*, *27*(1), 1–21. https://doi.org/10.1016/j.jfda.2018.12.002

He, X., Fu, P., Aker, W. G., & Hwang, H.-M. (2018). Toxicity of engineered nanomaterials mediated by nano-bio-eco interactions. *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews*, *36*(1), 21–42. https://doi.org/10.1080/10590501.2017.1418793

Hegeman, A. D. (2010). Plant metabolomics-meeting the analytical challenges of comprehensive metabolite analysis. *Briefings in Functional Genomics*, *9*(2), 139–148. https://doi.org/10.1093/bfgp/elp053

Hossain, Z., Mustafa, G., Sakata, K., & Komatsu, S. (2016). Insights into the proteomic response of soybean towards Al2O3, ZnO, and Ag nanoparticles stress. *Journal of Hazardous Materials*, *304*, 291–305. https://doi.org/10.1016/j.jhazmat.2015.10.071

Hounsome, N., Hounsome, B., Tomos, D., & Edwards-Jones, G. (2008). Plant metabolites and nutritional quality of vegetables. *Journal of Food Science*, *73*(4), R48–R65. https://doi.org/10.1111/j.1750-3841.2008.00716.x

Hu, J., Rampitsch, C., & Bykova, N. V. (2015). Advances in plant proteomics toward improvement of crop productivity and stress resistancex. *Frontiers in Plant Science*, *6*, 209. https://doi.org/10.3389/fpls.2015.00209

Huang, Y., Adeleye, A. S., Zhao, L., Minakova, A. S., Anumol, T., & Keller, A. A. (2019). Antioxidant response of cucumber (*Cucumis sativus*) exposed to nano copper pesticide: Quantitative determination via LC-MS/MS. *Food Chemistry*, *270*, 47–52. https://doi.org/10.1016/j.foodchem.2018.07.069

Huang, Y., Li, W., Minakova, A. S., Anumol, T., & Keller, A. A. (2018). Quantitative analysis of changes in amino acids levels for cucumber (*Cucumis sativus*) exposed to nano copper. *NanoImpact*, *12*, 9–17. https://doi.org/10.1016/j.impact.2018.08.008

Jacobson, A., Doxey, S., Potter, M., Adams, J., Britt, D., McManus, P., McLean, J., & Anderson, A. (2018). Interactions between a plant probiotic and nanoparticles on plant responses related to drought tolerance. *Industrial Biotechnology*, *14*(3), 148–156. https://doi.org/10.1089/ind.2017.0033

Jorge, T. F., Mata, A. T., & António, C. (2016). Mass spectrometry as a quantitative tool in plant metabolomics. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2079), 20150370. https://doi.org/10.1098/rsta.2015.0370

Judy, J. D., McNear, D. H., Chen, C., Lewis, R. W., Tsyusko, O. V., Bertsch, P. M., Rao, W., Stegemeier, J., Lowry, G. V., McGrath, S. P., Durenkamp, M., & Unrine, J. M. (2015). Nanomaterials in biosolids inhibit nodulation, shift microbial community composition, and result in increased metal uptake relative to bulk/dissolved metals. *Environmental Science & Technology*, *49*(14), 8751–8758. https://doi.org/10.1021/acs.est.5b01208

Kah, M., Kookana, R. S., Gogos, A., & Bucheli, T. D. (2018). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nature Nanotechnology*, *13*(8), 677–684. https://doi.org/10.1038/s41565-018-0131-1

Kah, M., Tufenkji, N., & White, J. C. (2019). Nano-enabled strategies to enhance crop nutrition and protection. *Nature Nanotechnology*, *14*(6), 532–540. https://doi.org/10.1038/s41565-019-0439-5

Keller, A. A., Huang, Y., & Nelson, J. (2018). Detection of nanoparticles in edible plant tissues exposed to nano-copper using single-particle ICP-MS. *Journal of Nanoparticle Research*, *20*(4), 101. https://doi.org/10.1007/s11051-018-4192-8

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. *Nature Protocols*, *5*(3), 536–549. https://doi.org/10.1038/nprot.2009.237

Komatsu, S., Mock, H.-P., Yang, P., & Svensson, B. (2013). Application of proteomics for improving crop protection/artificial regulation. *Frontiers in Plant Science*, *4*, 522–522. https://doi.org/10.3389/fpls.2013.00522

Kumar, A., Pathak, R. K., Gupta, S. M., Gaur, V. S., & Pandey, D. (2015). Systems biology for smart crops and agricultural innovation: Filling the gaps between genotype and phenotype for complex traits linked with robust agricultural productivity and sustainability. *Omics: A Journal of Integrative Biology*, *19*(10), 581–601. https://doi.org/10.1089/omi.2015.0106

Lai, Z. W., Yan, Y., Caruso, F., & Nice, E. C. (2012). Emerging techniques in proteomics for probing nano-bio interactions. *ACS Nano*, *6*(12), 10438–10448. https://doi.org/10.1021/nn3052499

Lu, W., Su, X., Klein, M. S., Lewis, I. A., Fiehn, O., & Rabinowitz, J. D. (2017). Metabolite measurement: Pitfalls to avoid and practices to follow. *Annual Review of Biochemistry*, *86*, 277–304. https://doi.org/10.1146/annurev-biochem-061516-044952

Ma, C., White, J. C., Dhankher, O. P., & Xing, B. (2015). Metal-based nanotoxicity and detoxification pathways in higher plants. *Environmental Science & Technology*, *49*(12), 7109–7122. https://doi.org/10.1021/acs.est.5b00685

Ma, C., White, J. C., Zhao, J., Zhao, Q., & Xing, B. (2018). Uptake of engineered nanoparticles by food crops: Characterization, mechanisms, and implications. *Annual Review of Food Science and Technology*, *9*(1), 129–153. https://doi.org/10.1146/annurev-food-030117-012657

Ma, X., Wang, Q., Rossi, L., Ebbs, S. D., & White, J. C. (2016). Multigenerational exposure to cerium oxide nanoparticles: Physiological and biochemical analysis reveals transmissible changes in rapid cycling *Brassica rapa*. *NanoImpact*, *1*, 46–54. https://doi.org/10.1016/j.impact.2016.04.001

MacLean, B., Tomazela, D. M., Shulman, N., Chambers, M., Finney, G. L., Frewen, B., Kern, R., Tabb, D. L., Liebler, D. C., & MacCoss, M. J. (2010). Skyline: An open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics (Oxford, England)*, *26*(7), 966–968. https://doi.org/10.1093/bioinformatics/btq054

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. (2015). Environmental effects of nanoceria on seed production of common bean (*Phaseolus vulgaris*): A proteomic analysis. *Environmental Science & Technology*, *49*(22), 13283–13293. https://doi.org/10.1021/acs.est.5b03452

Majumdar, S., Pagano, L., Wohlschlegel, J. A., Villani, M., Zappettini, A., White, J. C., & Keller, A. A. (2019). Proteomic, gene and metabolite characterization reveal the uptake and toxicity mechanisms of cadmium sulfide quantum dots in soybean plants. *Environmental Science: Nano*, *6*(10), 3010–3026. https://doi.org/10.1039/C9EN00599D

Majumdar, S., Peralta-Videa, J. R., Bandyopadhyay, S., Castillo-Michel, H., Hernandez-Viezcas, J.-A., Sahi, S., & Gardea-Torresdey, J. L. (2014). Exposure of cerium oxide nanoparticles to kidney bean shows disturbance in the plant defense mechanisms. *Journal of Hazardous Materials*, *278*, 279–287. https://doi.org/10.1016/j.jhazmat.2014.06.009

Majumdar, S., Peralta-Videa, J. R., Trujillo-Reyes, J., Sun, Y., Barrios, A. C., Niu, G., Margez, J. P. F., & Gardea-Torresdey, J. L. (2016). Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide

nanoparticles. *The Science of the Total Environment*, *569-570*, 201–211. https://doi.org/10.1016/j.scitotenv.2016.06.087

Majumdar, S., Trujillo-Reyes, J., Hernandez-Viezcas, J. A., White, J. C., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2016). Cerium biomagnification in a terrestrial food chain: Influence of particle size and growth stage. *Environmental Science & Technology*, *50*(13), 6782–6792. https://doi.org/10.1021/acs.est.5b04784

Matich, E. K., Chavez Soria, N. G., Aga, D. S., & Atilla-Gokcumen, G. E. (2019). Applications of metabolomics in assessing ecological effects of emerging contaminants and pollutants on plants. *Journal of Hazardous Materials*, *373*, 527–535. https://doi.org/10.1016/j.jhazmat.2019.02.084

Metch, J. W., Burrows, N. D., Murphy, C. J., Pruden, A., & Vikesland, P. J. (2018). Metagenomic analysis of microbial communities yields insight into impacts of nanoparticle design. *Nature Nanotechnology*, *13*(3), 253–259. https://doi.org/10.1038/s41565-017-0029-3

Mhlongo, M. I., Piater, L. A., Madala, N. E., Labuschagne, N., & Dubery, I. A. (2018). The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Frontiers in Plant Science*, *9*, 112. https://doi.org/10.3389/fpls.2018.00112

Mirzajani, F., Askari, H., Hamzelou, S., Schober, Y., Römpp, A., Ghassempour, A., & Spengler, B. (2014). Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L. *Ecotoxicology and Environmental Safety*, *108*, 335–339. https://doi.org/10.1016/j.ecoenv.2014.07.013

Mufamadi, M. S., & Sekhejane, P. R. (2017). Nanomaterial-based biosensors in agriculture application and accessibility in rural smallholding farms: Food security. In R. Prasad, M. Kumar, & V. Kumar (Eds.), *Nanotechnology: An agricultural paradigm* (pp. 263–278). Springer, Switzerland AG. https://doi.org/10.1007/978-981-10-4573-8_12

Nakabayashi, R., Hashimoto, K., Toyooka, K., & Saito, K. (2019). Keeping the shape of plant tissue for visualizing metabolite features in segmentation and correlation analysis of imaging mass spectrometry in *Asparagus officinalis*. *Metabolomics: Official Journal of the Metabolomic Society*, *15*(2), 24. https://doi.org/10.1007/s11306-019-1486-5

Nelson, B. C., Johnson, M. E., Walker, M. L., Riley, K. R., & Sims, C. M. (2016). Antioxidant cerium oxide nanoparticles in biology and medicine. *Antioxidants (Basel, Switzerland)*, *5*(2), 15. https://doi.org/10.3390/antiox5020015

Nhan, L. V., Ma, C., Rui, Y., Liu, S., Li, X., Xing, B., & Liu, L. (2015). Phytotoxic mechanism of nanoparticles: destruction of chloroplasts and vascular bundles and alteration of nutrient absorption. *Scientific Reports*, *5*, 11618. https://www.nature.com/articles/srep11618#supplementary-information https://doi.org/10.1038/srep11618

Peharec Štefanić, P., Jarnević, M., Cvjetko, P., Biba, R., Šikić, S., Tkalec, M., Cindrić, M., Letofsky-Papst, I., & Balen, B. (2019). Comparative proteomic study of phytotoxic effects of silver nanoparticles and silver ions on tobacco plants. *Environmental Science and Pollution Research International*, *26*(22), 22529–22550. https://doi.org/10.1007/s11356-019-05552-w

Pérez-Labrada, F., López-Vargas, E. R., Ortega-Ortiz, H., Cadenas-Pliego, G., Benavides-Mendoza, A., & Juárez-Maldonado, A. (2019). Responses of tomato plants under saline stress to foliar application of copper nanoparticles. *Plants (Basel, Switzerland)*, *8*(6), 151. https://doi.org/10.3390/plants8060151

Perez de Souza, L., Alseekh, S., Naake, T., & Fernie, A. (2019). Mass spectrometry-based untargeted plant metabolomics. *Current Protocols in Plant Biology*, *4*(4), e20100. https://doi.org/10.1002/cppb.20100

Pham, T.-H., Lee, B.-K., & Kim, J. (2016). Improved adsorption properties of a nano zeolite adsorbent toward toxic nitrophenols. *Process Safety and Environmental Protection*, *104*, 314–322. https://doi.org/10.1016/j.psep.2016.08.018

Piasecka, A., Kachlicki, P., & Stobiecki, M. (2019). Analytical methods for detection of plant metabolomes changes in response to biotic and abiotic stresses. *International Journal of Molecular Sciences*, *20*(2), 379. https://doi.org/10.3390/ijms20020379

Pinu, F. R., Beale, D. J., Paten, A. M., Kouremenos, K., Swarup, S., Schirra, H. J., & Wishart, D. (2019). Systems biology and multi-omics integration: Viewpoints from the metabolomics research community. *Metabolites*, *9*(4), 76. https://doi.org/10.3390/metabo9040076

Quanbeck, S., Brachova, L., Campbell, A., Guan, X., Perera, A., He, K., Rhee, S., Bais, P., Dickerson, J., Dixon, P., Wohlgemuth, G., Fiehn, O., Barkan, L., Lange, B. M., Lee, I., Cortes, D., Salazar, C., Shuman, J., Shulaev, V., … Nikolau, B. (2012). Metabolomics as a hypothesis-generating functional genomics tool for the annotation of arabidopsis thaliana genes of "unknown function". *Frontiers in Plant Science*, *3*, 15. https://doi.org/10.3389/fpls.2012.00015

Rabêlo, V. M., Magalhães, P. C., Bressanin, L. A., Carvalho, D. T., Reis, C. O. d., Karam, D., Doriguetto, A. C., Santos, M. H. d., Santos Filho, P. R. d S., & Souza, T. C. d. (2019). The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. *Scientific Reports*, *9*(1), 8164. https://doi.org/10.1038/s41598-019-44649-7

Ramírez-Sánchez, O., Pérez-Rodríguez, P., Delaye, L., & Tiessen, A. (2016). Plant proteins are smaller because they are encoded by fewer exons than animal proteins. *Genomics Proteomics Bioinformatics*, *14*(6), 357–370. https://doi.org/10.1016/j.gpb.2016.06.003

Rastogi, A., Tripathi, D. K., Yadav, S., Chauhan, D. K., Živčák, M., Ghorbanpour, M., El-Sheery, N. I., & Brestic, M. (2019). Application of silicon nanoparticles in agriculture. *3 Biotech*, *9*(3), 90. https://doi.org/10.1007/s13205-019-1626-7

Rawat, S., Pullagurala, V. L. R., Hernandez-Molina, M., Sun, Y., Niu, G., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2018). Impacts of copper oxide nanoparticles on bell pepper (*Capsicum annum* L.) plants: a full life cycle study. *Environmental Science: Nano*, *5*(1), 83–95. https://doi.org/10.1039/C7EN00697G

Rico, C. M., Barrios, A. C., Tan, W., Rubenecia, R., Lee, S., Varela-Ramirez, A., Peralta-Videa, J., & Gardea-Torresdey, J. (2015). Physiological and biochemical response of soil-grown barley (*Hordeum vulgare* L.) to cerium oxide nanoparticles. *Environmental Science and Pollution Research International*, *22*(14), 10551–10558. https://doi.org/10.1007/s11356-015-4243-y

Rico, C. M., Morales, M. I., Barrios, A. C., McCreary, R., Hong, J., Lee, W.-Y., Nunez, J., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2013). Effect of cerium oxide nanoparticles on the quality of rice (*Oryza sativa* L.) grains. *Journal of Agricultural and Food Chemistry*, *61*(47), 11278–11285. https://doi.org/10.1021/jf404046v

Rico, C. M., Wagner, D., Abolade, O., Lottes, B., & Coates, K. (2020). Metabolomics of wheat grains generationally-exposed to cerium oxide nanoparticles. *The Science of the Total Environment*, *712*, 136487. https://doi.org/10.1016/j.scitotenv.2019.136487

Rodrigues, A. M., Ribeiro-Barros, A. I., & António, C. (2019). Experimental design and sample preparation in forest tree metabolomics. *Metabolites*, *9*(12), 285. https://doi.org/10.3390/metabo9120285

Rodrigues, S. M., Demokritou, P., Dokoozlian, N., Hendren, C. O., Karn, B., Mauter, M. S., Sadik, O. A., Safarpour, M., Unrine, J. M., Viers, J., Welle, P., White, J. C., Wiesner,

M. R., & Lowry, G. V. (2017). Nanotechnology for sustainable food production: Promising opportunities and scientific challenges. *Environmental Science: Nano*, *4*(4), 767–781. https://doi.org/10.1039/C6EN00573J

Ruotolo, R., Maestri, E., Pagano, L., Marmiroli, M., White, J. C., & Marmiroli, N. (2018). Plant response to metal-containing engineered nanomaterials: An omics-based perspective. Environmental Science & Technology, *52*(5), 2451–2467. https://doi.org/10.1021/acs.est.7b04121

Salehi, H., Chehregani, A., Lucini, L., Majd, A., & Gholami, M. (2018). Morphological, proteomic and metabolomic insight into the effect of cerium dioxide nanoparticles to *Phaseolus vulgaris* L. under soil or foliar application. *The Science of the Total Environment*, *616-617*, 1540–1551. https://doi.org/10.1016/j.scitotenv.2017.10.159

Sanzari, I., Leone, A., & Ambrosone, A. (2019). Nanotechnology in plant science: To make a long story short [mini review]. *Frontiers in Bioengineering and Biotechnology*, *7*,120. https://doi.org/10.3389/fbioe.2019.00120

Schläpfer, P., Zhang, P., Wang, C., Kim, T., Banf, M., Chae, L., Dreher, K., Chavali, A. K., Nilo-Poyanco, R., Bernard, T., Kahn, D., & Rhee, S. Y. (2017). Genome-wide prediction of metabolic enzymes, pathways, and gene clusters in plants. *Plant Physiology*, *173*(4), 2041–2059. https://doi.org/10.1104/pp.16.01942

Singh, S., Vishwakarma, K., Singh, S., Sharma, S., Dubey, N. K., Singh, V. K., Liu, S., Tripathi, D. K., & Chauhan, D. K. (2017). Understanding the plant and nanoparticle interface at transcriptomic and proteomic level: A concentric overview. *Plant Gene*, *11*, 265–272. https://doi.org/10.1016/j.plgene.2017.03.006

Srivastava, A. K., Dev, A., & Karmakar, S. (2018). Nanosensors and nanobiosensors in food and agriculture. *Environmental Chemistry Letters*, *16*(1), 161–182. https://doi.org/10.1007/s10311-017-0674-7

Tiwari, M., Krishnamurthy, S., Shukla, D., Kiiskila, J., Jain, A., Datta, R., Sharma, N., & Sahi, S. V. (2016). Comparative transcriptome and proteome analysis to reveal the biosynthesis of gold nanoparticles in Arabidopsis. *Scientific Reports*, *6*, 21733. https://doi.org/10.1038/srep21733

Tumburu, L., Andersen, C. P., Rygiewicz, P. T., & Reichman, J. R. (2015). Phenotypic and genomic responses to titanium dioxide and cerium oxide nanoparticles in *Arabidopsis germinants*. Environmental Toxicology and Chemistry, *34*(1), 70–83. https://doi.org/10.1002/etc.2756

Unrine, J. M., Shoults-Wilson, W. A., Zhurbich, O., Bertsch, P. M., & Tsyusko, O. V. (2012). Trophic transfer of au nanoparticles from soil along a simulated terrestrial food chain. *Environmental Science & Technology*, *46*(17), 9753–9760. https://doi.org/10.1021/es3025325

Vannini, C., Domingo, G., Onelli, E., De Mattia, F., Bruni, I., Marsoni, M., & Bracale, M. (2014). Phytotoxic and genotoxic effects of silver nanoparticles exposure on germinating wheat seedlings. *Journal of Plant Physiology*, *171*(13), 1142–1148. https://doi.org/10.1016/j.jplph.2014.05.002

Vannini, C., Guido, D., Onelli, E., Prinsi, B., Marsoni, M., Espen, L., & Bracale, M. (2013). Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. *PLoS One*, *8*(7), e68752. https://doi.org/10.1371/journal.pone.0068752

Večeřová, K., Večeřa, Z., Dočekal, B., Oravec, M., Pompeiano, A., Tříska, J., & Urban, O. (2016). Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles. Environmental Pollution (Barking, ESSEX: 1987), *218*, 207–218. https://doi.org/10.1016/j.envpol.2016.05.013

Verano-Braga, T., Miethling-Graff, R., Wojdyla, K., Rogowska-Wrzesinska, A., Brewer, J. R., Erdmann, H., & Kjeldsen, F. (2014). Insights into the cellular response triggered by silver nanoparticles using quantitative proteomics. *ACS Nano*, *8*(3), 2161–2175. https://doi.org/10.1021/nn4050744

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. (2018). Ecological risk assessment of nano-enabled pesticides: A perspective on problem formulation. *Journal of Agricultural and Food Chemistry*, *66*(26), 6480–6486. https://doi.org/10.1021/acs.jafc.7b02373

Wang, A., Jin, Q., Xu, X., Miao, A., White, J. C., Gardea-Torresdey, J. L., Ji, R., & Zhao, L. (2020). High-throughput screening for engineered nanoparticles that enhance photosynthesis using mesophyll protoplasts. *Journal of Agricultural and Food Chemistry*, *68*(11), 3382–3389. https://doi.org/10.1021/acs.jafc.9b06429

Wang, W., Tai, F., & Chen, S. (2008). Optimizing protein extraction from plant tissues for enhanced proteomics analysis. *Journal of Separation Science*, *31*(11), 2032–2039. https://doi.org/10.1002/jssc.200800087

Wu, B., Zhu, L., & Le, X. C. (2017). Metabolomics analysis of TiO2 nanoparticles induced toxicological effects on rice (*Oryza sativa* L.). *Environmental Pollution (Barking, Essex: 1987))*, *230*, 302–310. https://doi.org/10.1016/j.envpol.2017.06.062

Wu, H., Tito, N., & Giraldo, J. P. (2017). Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. *ACS Nano*, *11*(11), 11283–11297. https://doi.org/10.1021/acsnano.7b05723

Xu, J., & Wang, N. (2019). Where are we going with genomics in plant pathogenic bacteria? *Genomics*, *111*(4), 729–736. https://doi.org/10.1016/j.ygeno.2018.04.011

Yin, J., Wang, Y., & Gilbertson, L. M. (2018). Opportunities to advance sustainable design of nano-enabled agriculture identified through a literature review [10.1039/C7EN00766C. *Environmental Science: Nano*, *5*(1), 11–26. https://doi.org/10.1039/C7EN00766C

Yuvaraj, M., & Subramanian, K. S. (2018). Development of slow release Zn fertilizer using nano-zeolite as carrier. *Journal of Plant Nutrition*, *41*(3), 311–320. https://doi.org/10.1080/01904167.2017.1381729

Zhang, H., Du, W., Peralta-Videa, J. R., Gardea-Torresdey, J. L., White, J. C., Keller, A., Guo, H., Ji, R., & Zhao, L. (2018). Metabolomics Reveals How cucumber (*Cucumis sativus*) reprograms metabolites to cope with silver ions and silver nanoparticle-induced oxidative stress. *Environmental Science & Technology*, *52*(14), 8016–8026. https://doi.org/10.1021/acs.est.8b02440

Zhao, L., Hu, J., Huang, Y., Wang, H., Adeleye, A., Ortiz, C., & Keller, A. A. (2017). 1H NMR and GC-MS based metabolomics reveal nano-Cu altered cucumber (*Cucumis sativus*) fruit nutritional supply . *Plant Physiology and Biochemistry: PPB*, *110*, 138–146. https://doi.org/10.1016/j.plaphy.2016.02.010

Zhao, L., Hu, Q., Huang, Y., Fulton, A. N., Hannah-Bick, C., Adeleye, A. S., & Keller, A. A. (2017). Activation of antioxidant and detoxification gene expression in cucumber plants exposed to a Cu(OH)2 nanopesticide. *Environmental Science: Nano*, *4*(8), 1750–1760. https://doi.org/10.1039/C7EN00358G

Zhao, L., Huang, Y., Adeleye, A. S., & Keller, A. A. (2017). Metabolomics reveals Cu(OH)2 nanopesticide-activated anti-oxidative pathways and decreased beneficial antioxidants in spinach leaves. *Environmental Science & Technology*, *51*(17), 10184–10194. https://doi.org/10.1021/acs.est.7b02163

Zhao, L., Huang, Y., Hannah-Bick, C., Fulton, A. N., & Keller, A. A. (2016). Application of metabolomics to assess the impact of Cu(OH)2 nanopesticide on the nutritional value of

lettuce (*Lactuca sativa*): Enhanced Cu intake and reduced antioxidants. *NanoImpact*, *3-4*, 58–66. https://doi.org/10.1016/j.impact.2016.08.005

Zhao, L., Huang, Y., Hu, J., Zhou, H., Adeleye, A. S., & Keller, A. A. (2016). (1)H NMR and GC-MS based metabolomics reveal defense and detoxification mechanism of cucumber plant under Nano-Cu stress. *Environmental Science & Technology*, *50*(4), 2000–2010. https://doi.org/10.1021/acs.est.5b05011

Zhao, L., Huang, Y., & Keller, A. A. (2018). Comparative metabolic response between cucumber (*Cucumis sativus*) and corn (*Zea mays*) to a Cu(OH)2 nanopesticide. *Journal of Agricultural and Food Chemistry*, *66*(26), 6628–6636. https://doi.org/10.1021/acs.jafc.7b01306

Zhao, L., Huang, Y., Paglia, K., Vaniya, A., Wancewicz, B., & Keller, A. A. (2018). Metabolomics reveals the molecular mechanisms of copper induced cucumber leaf (*Cucumis sativus*) senescence. *Environmental Science & Technology*, *52*(12), 7092–7100. https://doi.org/10.1021/acs.est.8b00742

Zhao, L., Huang, Y., Zhou, H., Adeleye, A. S., Wang, H., Ortiz, C., Mazer, S. J., & Keller, A. A. (2016). GC-TOF-MS based metabolomics and ICP-MS based metallomics of cucumber (*Cucumis sativus*) fruits reveal alteration of metabolites profile and biological pathway disruption induced by nano copper. *Environmental Science: Nano*, *3*(5), 1114–1123. https://doi.org/10.1039/C6EN00093B

Zhao, L., Ortiz, C., Adeleye, A. S., Hu, Q., Zhou, H., Huang, Y., & Keller, A. A. (2016). Metabolomics to detect response of lettuce (*Lactuca sativa*) to Cu(OH)2 nanopesticides: oxidative stress response and detoxification mechanisms. *Environmental Science & Technology*, *50*(17), 9697–9707. https://doi.org/10.1021/acs.est.6b02763

Zhao, L., Sun, Y., Hernandez-Viezcas, J. A., Hong, J., Majumdar, S., Niu, G., Duarte-Gardea, M., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2015). Monitoring the environmental effects of CeO2 and ZnO nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ μ-XRF mapping of nutrients in kernels. *Environmental Science & Technology*, *49*(5), 2921–2928. https://doi.org/10.1021/es5060226

Zhao, L., Zhang, H., Wang, J., Tian, L., Li, F., Liu, S., Peralta-Videa, J. R., Gardea-Torresdey, J. L., White, J. C., Huang, Y., Keller, A., & Ji, R. (2019). C60 fullerols enhance copper toxicity and alter the leaf metabolite and protein profile in cucumber. *Environmental Science & Technology*, *53*(4), 2171–2180. https://doi.org/10.1021/acs.est.8b06758

Zhao, L., Zhang, H., White, J. C., Chen, X., Li, H., Qu, X., & Ji, R. (2019). Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. *Environmental Science: Nano*, *6*(6), 1716–1727. https://doi.org/10.1039/C9EN00137A

Zhao, X., Cui, H., Wang, Y., Sun, C., Cui, B., & Zeng, Z. (2018). Development strategies and prospects of nano-based smart pesticide formulation. *Journal of Agricultural and Food Chemistry*, *66*(26), 6504–6512. https://doi.org/10.1021/acs.jafc.7b02004

Zulfiqar, F., Navarro, M., Ashraf, M., Akram, N. A., & Munné-Bosch, S. (2019). Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Plant Science : An International Journal of Experimental Plant Biology*, *289*, 110270. https://doi.org/10.1016/j.plantsci.2019.110270