



**BITS AND PIECES:  
AN ANTHOLOGY OF  
PROSE WRITINGS**

*Edited by*  
**Vinod Selastin  
Jennifer Fernandez  
Cynthia Catherine Michael**

A photograph of a library bookshelf filled with books. The books are arranged on several shelves, and the lighting is warm, creating a cozy atmosphere. The text is overlaid on the bottom part of the image.

Postgraduate and Research Department of English  
FATIMA MATA NATIONAL COLLEGE (AUTONOMOUS), KOLLAM

# **LITERARY DIMENSIONS: Indian Writing in English**

Core Text for Semester V First Degree  
Programme under CBCSS for BA English  
Language and Literature and BA English  
Language, Literature and Communication.

Eds

Dr Cynthia Catherine Michael  
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Dr Y. Mercy Famila  
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Postgraduate and Research Department of English

FATIMA MATA NATIONAL COLLEGE  
(AUTONOMOUS), KOLLAM

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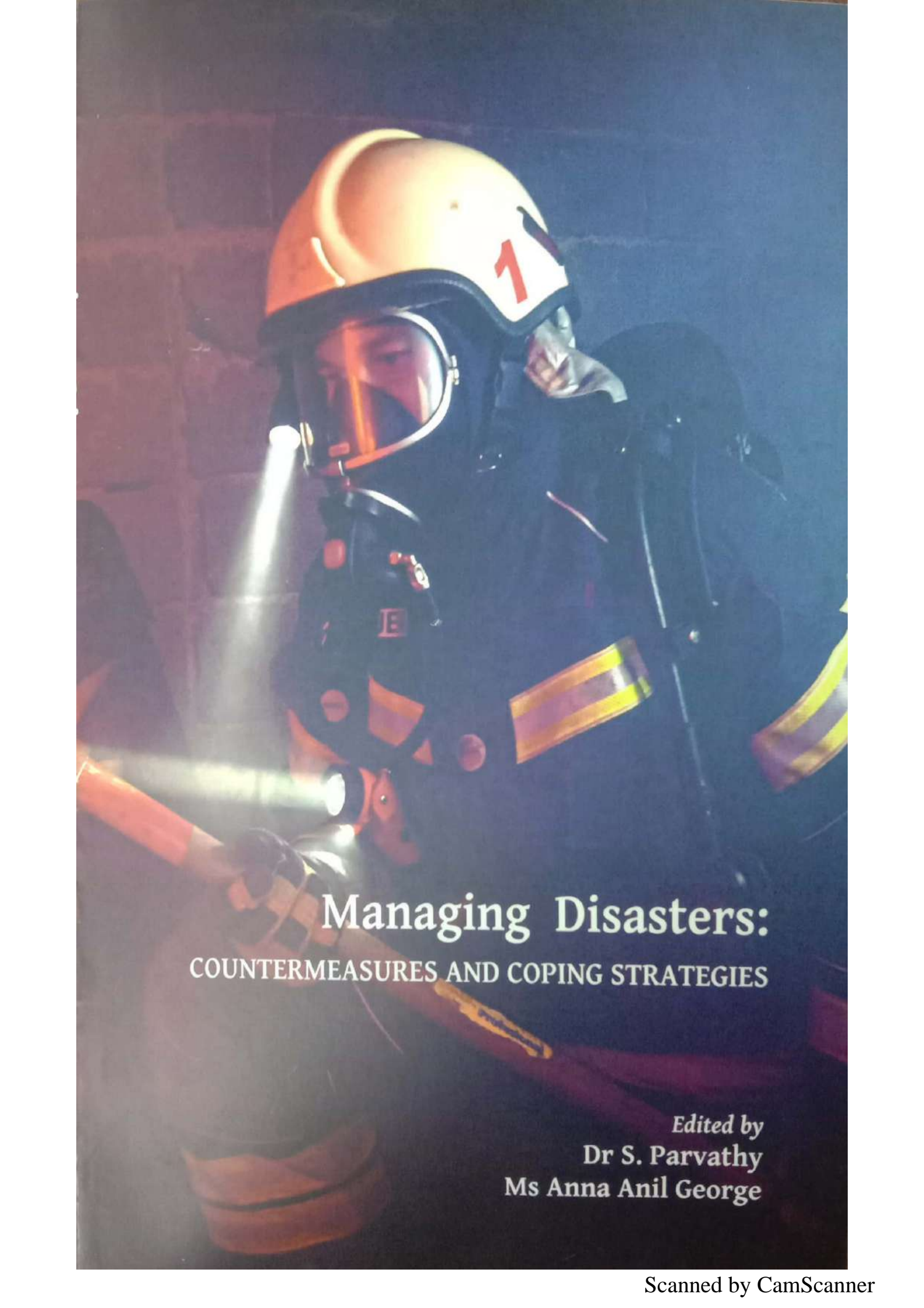
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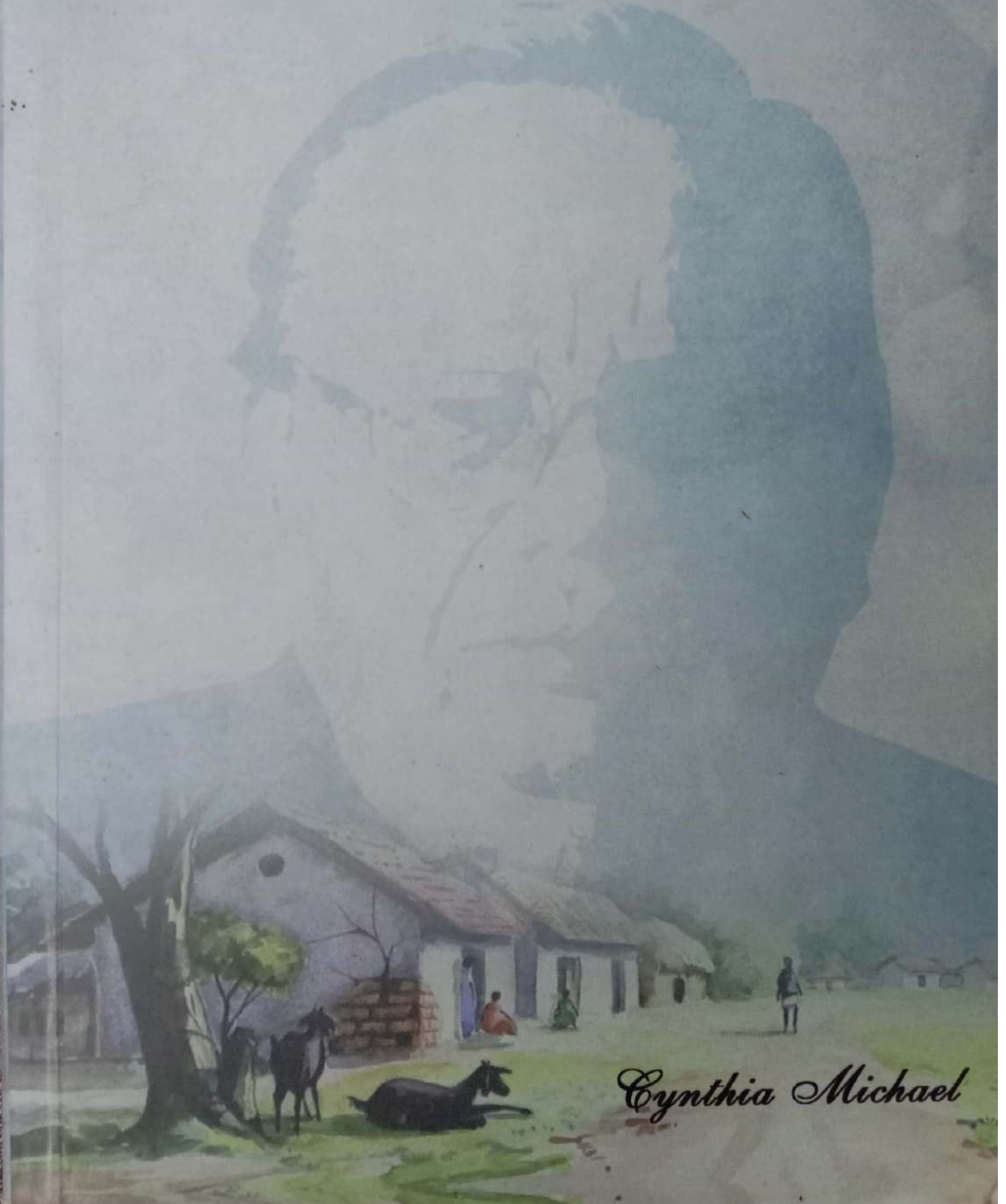


Postgraduate and Research Department of English

FATIMA MATA NATIONAL COLLEGE (AUTONOMOUS), KOLLAM

# BECOMING INDIAN

Unravelling the world of Ruskin Bond



*Cynthia Michael*



Dr. Cynthia Catherine Michael was born to teachers Sahayan Michael and late Bridget Michael in Kollam, Kerala, India. Her early schooling was in Jamaica and Nigeria. She completed her Post Graduation in English Language and Literature from Fatima Mata National College, Kollam. She received her Ph.D degree from the University of Kerala, India. Presently she is working as an Associate Professor of English in Fatima Mata National College. The author is married to Dr. Sanil Sebastian and blessed with three children Irena, Ryan and Adrian.

This book has very definite value for Indian researchers, fiction lovers, prose lovers and all interested in colonialism and post colonialism. Study of some of the key novels and works of Ruskin Bond show the true sense of being Indian. Its value lies in showing that West and East can meet. Bond also transcends both Rudyard Kipling and E M. Forster. The historical and geographical aspect is also interesting as it involves the ideological and psychological analysis. Bond's world view is shown to be unique as is his philosophy. His definition of being is essentially democratic, secular, liberal and humanist.

-Dr. Koshy A V

Dr. Koshy A V, academician, critic, poet editor and anthologist, author of books like Samuel Beckett's English Poetry: Transcending the Roots of Resistance in Language and many other books of literary criticism, theory and poetry and presently Assistant Professor in the English Department, College of Arts and Humanities for Women, Jazan University, Saudi Arabia.



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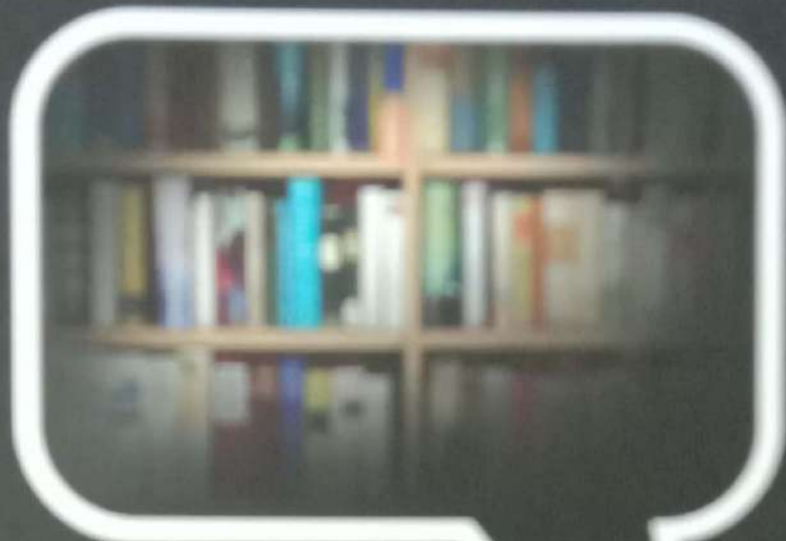
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# Realms of Literature

AN ANTHOLOGY OF SHORT FICTION  
AND ONE-ACT PLAYS

EDITED BY  
Dr. Cynthia Catherine Walker  
Kavita Chatterjee  
Rishabh

# Realms of Literature

AN ANTHOLOGY OF SHORT FICTION  
AND ONE ACT PLAYS

The book *Realms of Literature: An Anthology of Short Fiction and One Act Plays* has been prepared as an introductory textbook for the courses "Reading Fiction" and "Reading Drama" for the students of B.A. English Language and Literature and B.A. English Language, Literature and Communication of Fatima Mata National College (Autonomous), Kollam, Kerala. This anthology endeavors students to explore the world of drama and fiction. The learners are introduced to some of the best works of short fiction and one act plays written by authors of different nationalities. They are to understand with empathy, the world view of others as well as the ways in which they reimagine life.

For the classroom, each chapter has an introduction, glossary and comprehension section. This will inspire students to research the multi-faceted nature of literature in general and prepare them for the examination, from an analysis point of view by stimulating them to study in depth.

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# Chapter 4

## Response to Engineered Nanomaterials in *Arabidopsis thaliana*, a Model Plant



Sinilal Bhaskaran and Shivendra Sahi

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## 1 Introduction

Nanomaterials are diverse in the chemical composition, size, shape, surface characteristics, purity, stability and optical, thermal and electrical properties and have gained manifold applications in the modern industrial society. Their interactions with living organisms are significant because of the increased permeability conferred by the small size. The property of target-specific, controlled release of nanomaterials is utilized to deliver a variety of molecules into plant and animal cells. In plants, nano fertilizers have been advocated for enhancement of nutrient-use efficiency by the controlled release in meeting the plant's demands (Zulfiqar et al. 2019). This application in turn prevents nutrient loss through run-away water and transformation to chemical forms that are not consumable to plants. Pesticide application based on nanotechnology helps to reduce the dosage and frequency of application (Hayles et al. 2017; Ojha et al. 2018). The resultant drastic reduction in the usage of fertilizers and plant protectants anticipates lower investment and harm to the environment (Adisa et al. 2019). Biosensor technology using nanomaterials is underway for detection of plant pathogens and plant metabolic flux (Chaudhry et al. 2018). Some nanomaterials are used for targeted delivery of DNA, but their share is negligible compared to other uses (Riley and

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Vermerris 2017). Because of the desirable physical and chemical properties mentioned above, new applications are on way every day; at the same time, concern over the uncontrolled release of nanomaterials into the environment is soaring in scientific community. Before a novel nanomaterial enters the human supply chain, their entry into plants from air, water and soil and how they interact with plant cells *in vivo* need to be scrutinized as they can be transferred to next trophic levels affecting the ecosystem. Unfortunately, most of the applications of nanomaterials have not undergone such scrutinizes.

Attempts to understand the interaction of nanomaterials tested *in vitro* and *in vivo* narrate both stimulatory and inhibitory effects. In plants, the changes occurring in morphology, anatomy, physiology and gene expression as a result of exposure to nanomaterials are usually observed to assess the impact. Crop species have been studied mostly to explore the intended outcomes of specific applications. However, the limited information on physiology and gene interactions of nanomaterials in most of the species poses a question about the depth and reliability of results. On the other hand, results of the studies conducted in experimental/ model organisms are more informative because of several reasons. This includes the ease in conducting experiments in controlled conditions, analysis using standard protocols, reproducibility in results and the possibilities for exploring molecular mechanisms using the techniques of genomics and metabolomics due to the availability of complete genome sequence (Montes et al. 2017). *Arabidopsis thaliana* is preferred over other model plants because of its small size enabling the growth of large number of plants in a small area, short life cycle, self-fertile nature and high potential for producing mass of seeds. The easier transformation using *Agrobacterium tumefaciens*, availability of gene overexpression and mutant lines, small diploid genome and the public availability of its resources convenient for genetics and genomics analyses make *Arabidopsis* system ideal for nanointeractions. Realizing the merits of the system, *A. thaliana* has been exposed to a range of nanomaterials for an in-depth characterization of the effects, which are discussed below. For convenience, the impact of different nanomaterials by virtue of their chemical compositions is discussed here, giving emphasis to the morphological/anatomical, physiological and genetic effects.

**Gold:** Influence of gold nanoparticles (AuNPs) on growth of *Arabidopsis* has been characterized by seed germination and its percentage, measuring fresh weight and dry weight, root growth by increase in root length, branching, etc. Both inhibitory and promotive effects have been reported. Low concentrations promoted growth, exhibiting about twofold increase in the percentage of seed germination (Kumar et al. 2013). Growth promotion was also observed in terms of shoot, root length, number of lateral roots and rosette leaves bringing an increase in fresh weight ranging from three- to sixfold. Increase in water content was also noticed in the treated plants, which imparted the increase in fresh weight. The overall growth promotive effects were found persistent throughout the life of the plants evidenced by early flowering and increase in yield even after retraction from exposure for 15 days. In a similar study, AuNP treatment (100 mg/L) significantly reduced the number and length of lateral roots at a high concentration and the effect was irrespective of the particle shapes (Siegel et al. 2018). However, size influenced growth as inhibition of primary root and promotion of root hair growth in the experimental

group treated with AuNPs of 10 nm. Surface charge of the particles was also observed to be influencing growth (Hendel et al. 2019). Compared to neutral AuNPs, the charged ones reduced growth of root meristematic region. Negatively charged AuNPs induced root hair and lateral root growth.

AuNPs enter plants through the root system and get transported to other parts of the plant via vascular system (Koo et al. 2015a, b). Analysis of the surface charge of AuNPs and its role in absorption indicated the preference of negatively charged AuNPs over those having positive charge (Avellan et al. 2017). Particles were lodged in the border cells and the mucilage secreted by the cells at the root tip. Majority of the positively charged particles were found accumulated outside and a few spotted inside were considered internalized by the process of endocytosis. In addition to the surface charge, size also influenced uptake and translocation. Surprisingly, a similar study conducted using positively, negatively and neutral AuNPs came up with contradictory results (Hendel et al. 2019). Neutral AuNPs induced vacuolization in the rhizodermal and cortical cells at the root tip which is a typical response to heavy metals. Regardless of the surface charge, AuNPs were found accumulated in the vicinity to the root surface. Treatment with neutral and positively charged particles stimulated the detachment of plasma membrane and the space formed was found filled with a secretion which normally occurs in case of abiotic stress. Even though negatively charged AuNPs did not impose membrane detachment, increase in cell wall thickness was observed which is to be considered as a mechanism to prevent their entry. Charge on the particles facilitated their entry into protoplasts. Positively charged AuNPs were favoured than the latter. Both endocytotic and non-endocytotic modes of entries were observed in this study. Thus, it is clear that the studies conducted so far in *Arabidopsis* are focused mainly on the morphological and anatomical changes. Few of them tried to understand the mechanism of entry, transport and accumulation. Monitoring physiological changes can give some idea on the metabolic pathways interacting with AuNPs. Analyses of few antioxidant enzyme activities and the expression of few microRNAs conclude that the changes observed were triggered by the related micro RNAs (Kumar et al. 2013). AuNPs accumulated inside plant tissues generate heat according to an analysis using thermal imaging. High induction of heat shock proteins associated with the condition is an indication of that (Koo et al. 2015a, b). Stress symptoms like reduction of chlorophyll and formation of anthocyanin were accompanied with the changes in test groups fed with higher AuNP concentrations (Wang et al. 2013). So, it is evident that a comprehensive study of the mechanism of interaction of AuNPs in *Arabidopsis* is yet to come. *Arabidopsis* can be effectively utilized to unravel the molecular interactions of nanomaterials based on its available genomic information.

Apart from being utilized as an agent for molecular delivery in plants, AuNPs themselves can be synthesized by plants and plant products (Shankar et al. 2004). Green engineering approach for nanofabrication is receiving attention in light of the nanoparticles produced with novel surface coatings and the environment-friendly nature of the process. Another application of such nanomaterials could be directed to phytoextraction of soil pollutants or in the water filtration systems. In plant-inspired process, gold is fed to growing plants as water-soluble salts that transform

to gold nanoparticles after reacting with plant chemicals *in vivo*. The regulatory points of the redox reactions involved are yet to be deciphered for a smooth production cycle. Further, the ions that are potentially reactive may be translocated to different parts of the plant by metal transporter proteins and get converted to the metallic form by certain other reducing agents to make them unreactive (Taylor et al. 2014; Jain et al. 2014). Tiwari et al. (2016) reported majority of metal responsive and binding genes were upregulated when plants were exposed to gold salts ( $\text{KAuCl}_4$ ) forming a bulk of AuNPs in their root and shoot tissues. Among differentially expressed genes, 70 genes were upregulated up to twofold in root (Fig. 4.1; Table 4.1). The classification of upregulated genes based on metal responses indicates that 12.46% genes were associated with cation binding. The expression of ferric reduction oxidase 5 (FRO5) was highest (17.53-fold) among upregulated genes (Fig. 4.1; Table 4.1). The induced loci encode different types of transporters such as copper transporter, nitrate transporter, ABC transporter, heavy-metal-associated protein (HMA), zinc (Zn) transporter, malate transporter and phosphate transporter. These genes are responsible for uptake of essential elements and nutrients such as Fe, Cu, Zn,  $\text{NO}_3$  and  $\text{PO}_4$ .

**Silver:** Silver nanoparticles (AgNPs) occupy a major part of the commercially utilized nanoparticles. They are available as coated and uncoated forms. Their interaction when studied by supplying 0.01 to 100 mg/L to Arabidopsis demonstrated dose-dependent effects (Wang et al. 2013). Like gold nanoparticles, lower concentrations of AgNPs enhanced growth while the higher concentrations were found inhibitory. Parameters of growth like size of rosette leaves, root length and shoot weight exhibited the same distinct patterns across the different concentrations tested. Smaller nanoparticles were found to be more toxic in this investigation. The results obtained were comparable to those of  $\text{AgNO}_3$  that produced AgNPs inside plant tissues, largely in leaves. In a similar study conducted with AgNPs and  $\text{Ag}^+$ , more or less similar patterns in the growth parameters were observed in the concentrations treated (Kaveh et al. 2013). Silver concentration was found higher in plants exposed to  $\text{AgNO}_3$  attesting the higher permeability of silver ions than their particles. AgNPs and  $\text{Ag}^+$  were inhibitory to root growth and were of the same toxic responses (Baghkheirati and Lee 2015). In another study, a comparison between the effects of AgNPs and  $\text{Ag}^+$  demonstrated higher interferences of AgNPs in growth, reproduction and metabolism (Ke et al. 2018). Shoot and root growth were inhibited. Reduction in chlorophyll and the production of anthocyanin in leaves indicated stress. Decrease in reproductive efficiency was observed which was marked by the reduction in bolting height, bud number, number of pods, pod length and their biomass. Notwithstanding that sugar, phenylpropanoid and amino acid pathways were affected by both AgNPs and  $\text{Ag}^+$ , enhancement of galactose metabolism and the reduction in the levels of amino acids valine, serine and aspartate were characteristic to AgNPs. Stretching of the vegetative growth is another effect induced which resulted in shortening of the reproductive phase followed by a poor regeneration capacity of the seeds (Geisler-Lee et al. 2014). Particles were found accumulated inside the root cells, vascular tissues, cotyledons and stomata at different days after the treatment. Toxicity affected meristematic cells at the root tips preventing the



**Fig. 4.1** Plant gene set enrichment analysis of significantly upregulated genes when *Arabidopsis* seedlings were exposed to 10 ppm  $\text{KAuCl}_4$  (Source: Tiwari et al. (2016): *Scientific Reports*, 6:21733, DOI: <https://doi.org/10.1038/srep21733>)

**Table 4.1** Comparison of microarray data by quantitative RT-PCR (Source: Tiwari et al. (2016): Scientific Reports, 6:21733, DOI: <https://doi.org/10.1038/srep21733>)

Locus ID	Annotation	Microarray	qRT-PCR	
			10 ppm Au	25 ppm Au
AT5G23990	FRO5	17.54	7.269	1.855
AT2G46830	CCA1	3.88	4.645	0.472
AT1G63950	HMA3	3.833	26.459	0.025
AT3G28345	ABC transporter B family member	3.102	2.215	0.526
AT5G14570	High affinity nitrate transporter	3.4	2.250	2.406
AT3G46900	COPT2	4.22	4.835	1.192
AT1G01060	LHY	3.59	2.978	1.638
AT3G12320	Light-inducible and clock-regulated 3, LNK3	4.81	8.889	0.498
AT1G33730	CYP76C5	3.77	1.685	2.308
AT5G17300	RVE1	2.919	2.689	1.986
AT5G24380	YSL2	3	1.332	0.386
AT3G09600	Myb	2.37	2.087	2.288
AT4G10530	Subtilase family protein	-6.85	0.073	0.450
AT5G49770	Leucine-rich repeat receptor-like protein kinase	-2.79	0.183	0.385
AT2G44840	ERF13	-2.01	0.179	1.116

growth of primary and lateral roots. Localization using isotopic labelling and single particle Inductively Coupled Plasma (ICP) analysis detected the particles in roots and their transport to the other parts (Nath et al. 2018). On analysis using single-cell ICP, the particles were found predominantly at the middle lamella and cell walls of the root and a smaller portion found translocated to other parts of the plant; however those detected inside the cells were aggregated (Bao et al. 2016). Exposure of seedlings to 0.2–1 mg/L AgNPs for a maximum period of 72 hrs resulted in detecting silver inside the plant tissues with the help of ICP analysis (Nair and Chung 2014a). Since toxicity has been reported in other plant and animal systems, AgNP usage cannot be considered to be safe. Changes in physiological parameters like reduction in chlorophyll content, increase in anthocyanin, etc. were observed when seedlings were exposed to the above concentrations of AgNPs for 14 days (Nair and Chung 2014b) as observed in an earlier study (Wang et al. 2013). Increase in lipid peroxidation, Reactive Oxygen Species (ROS) production and change in mitochondrial membrane potential were recorded in roots of the seedlings exposed. Shape of the particles also influences the responses in plants (Syu et al. 2014). Spherical particles induced anthocyanin production and high level of Super Oxide Dismutase (SOD) indicating their inhibitory nature. Whereas decahedral particles promoted root growth and were recognized as the one produced the lowest level of SOD. Expression of proteins associated with ROS accumulation and cell proliferation were found common in all types. Stress induced by AgNPs in plants when compared to that of cold, salt, drought and heat stresses were found different and milder (Baghkeirati and Lee 2015). ROS generation was found common in all. The stress induced by



AgNPs was observed to be having highest similarity with cold stress compared to the others. AgNPs above 300 mg/L were found to be interfering with potassium (K<sup>+</sup>) efflux and calcium (Ca<sub>2</sub><sup>+</sup>) influx impairing the transport through plasma membrane (Sosan et al. 2016). This study also noticed reduction of root growth, photosynthesis rate and the formation of ROS as reported in several other studies conducted in *Arabidopsis*. Oxidation of apoplastic L-ascorbic acid was also observed and annotated as an effect of AgNPs.

Significant upregulation of the genes involved in glutathione (GSH) biosynthesis on AgNP- exposure clearly demonstrated the toxic interferences of AgNPs in plants (Nair and Chung 2014b). Gene expression patterns in response to AgNPs were found similar to those in response to fungal infection and anion transport. The two major categories of genes responded were of oxidative stress and cell proliferation. An overlap in gene expression pattern was visible in response to AgNO<sub>3</sub> and AgNPs (Kaveh et al. 2013). Cell cycle regulating genes *AtPCNA1* and *AtPCNA2* were upregulated up to 72 hrs and observed to be downregulated after that. DNA mismatch repair genes *AtMSH2*, *AtMSH3*, *AtMSH6* and *AtMSH7* showed downregulation in the plants exposed (Nair and Chung 2014a, b, c). Gene expression results also revealed the prominent role of systemic signalling in toxic responses exhibited by the plant. Most of the comparative studies using AgNPs and Ag<sup>+</sup> performed for distinguishing their effects separately concluded that the toxic effects of AgNPs are because of the Ag<sup>+</sup> ions released from the particles. However, in a study using equivalent concentrations of the two, both were found producing similar effects denying the chances for Ag<sup>+</sup> ions to interfere (Zhang et al. 2019). Both induced ROS accumulation, reduction in efficiency of photosynthesis, and showed similarity in gene expression pattern. Genes associated with photosynthesis, oxidative stress, signal perception and response, etc. were found differentially expressed. Genes involved in the synthesis of Glucosinolates, the group of secondary metabolites were identified as specifically regulated by AgNPs, designating the triggering of defence mechanisms.

Nanoparticles are often used for delivery of other molecules that surface-bond with the particles, and their individual effects have to be differentiated from those of the combinations. Such an evaluation conducted for the conjugate of herbicide Imazethapyr with AgNPs demonstrates enhancement of the toxicity (Wen et al. 2016). AgNPs alone at 25 μM and 50 μM enhanced plant growth, but decreased chlorophyll content. However, the treatment did not increase the free amino acids as observed in the responses associated with heavy metal exposure. When leaves of the AgNP-treated plants were examined, amino acid content increase was noticed, and it was later confirmed due to the release of Ag<sup>+</sup> ions from the AgNPs. Outer surface of the roots was covered with AgNPs and their concentration was much less inside attesting their formation in conjunction with the Ag<sup>+</sup> moved inside. As observed in several other studies, ROS was not detected upon staining in the AgNP-treated group, but increased activities of detoxifying enzymes SOD and Catalase (CAT) were recorded. Toxicity observed was higher in the experimental group subjected to the herbicide alone and was still higher with the AgNP-herbicide conjugate. Inhibition of geotropic root growth was observed in response to high concentration

of AgNP exposure (300 ng/L) and was identified to be because of the inhibition in auxin synthesis (Sun et al. 2017). Since expression of the concerned receptors was found downregulated and *AFB4*, a negative regulator of auxin signalling, as upregulated, the chances for blockade of auxin signalling were predicted. It might be occurring through the physical blockade created by the nanoparticles lodged at the intercellular spaces and inside the cells. The conjugate of herbicide Diclofop-methyl with AgNPs when used in *Arabidopsis* showed less inhibitory effects than AgNPs used alone (Li et al. 2018). Growth inhibition, increase in anthocyanin, accumulation of  $H_2O_2$  and decrease in the rate of photosynthesis were observed in the experimental group with AgNP alone. However, the conjugate had reduced values in these parameters, which is presumed to occur due to the low stability of the  $Ag^+$  ions released from the AgNPs. When particles of two sizes (10 and 60 nm) were exposed to the plants, most of them found aggregated on root surface and a very small percentage distributed inside (Wang et al. 2019).

**Copper:** Copper oxide nanoparticles (CuONPs) are not directly being utilized for applications related to agriculture. However, they have several other industrial applications because of their catalytic activity. Most of the studies conducted in *Arabidopsis* to assess the interaction of CuONPs were carried out along with  $Cu^+$  for differentiating individual effects. Since the release of metal ions from nanoparticles observed in the case of few others, studying the two in parallel and comparing the effects can identify the specific effects of CuONPs. These particles are observed to be inhibiting growth, effected by reduction in biomass and inhibition of root growth (Tang et al. 2016; Wang et al. 2016a, b; Yuan et al. 2016; Landa et al. 2017; Zhao et al. 2018). On the contrary, the particles were found less toxic than  $Cu^+$  in a similar investigation done in *Arabidopsis* (Ke et al. 2017). Loss of root gravitropism also has reported in a study conducted only with CuONPs (Nair and Chung 2014c). CuONPs were mainly found accumulated on the root surface and cell walls (Yuan et al. 2016) and induced changes in anatomy of the root, mainly manifested as lignin deposition, increase in width of cells and swelling at the root elongation zone (Wang et al. 2016a, b). Cells at root tips died due to the toxicity (Nair and Chung 2014c; Tang et al. 2016). A small portion of the particles were localized in aerial parts (Soria et al. 2019), which induced vacuole shrinkage and cell death (Yuan et al. 2016). Major physiological changes were generation of reactive oxygen species (Nair and Chung 2014c; Tang et al. 2016; Wang et al. 2016a, b; Yuan et al. 2016) and increase in anthocyanin (Nair and Chung 2014c; Ke et al. 2017). Increase in saturated fatty acids and decrease in unsaturated fatty acids lead to collapse of membranes (Yuan et al. 2016). CuONPs triggered the differential expression of a subset of genes regulating cell division and stress response. SOD, CAT and Peroxidase (PRX) were expressed in accordance with the ROS generated (Nair and Chung 2014c; Tang et al. 2016; Wang et al. 2016a, b; Landa et al. 2017). Auxin responsive genes (Wang et al. 2016a, b) and lignin biosynthesis related genes (Tang et al. 2016) were found upregulated. Stress induction and response was found triggered because of the changes in genes involved in proline biosynthesis and sulphur assimilation (Nair and Chung 2014c), heat shock proteins, methionine synthesis (Tang et al. 2016), metallo chaperonins

and water deficiency response (Landa et al. 2017). Metabolite profiling identified the increase in isothiocyanates, scopoletin and jasmonates which are functioning in defence signalling and response (Soria et al. 2019).

**Zinc:** Zinc oxide nanoparticles (ZnONPs) are extensively used in cosmetics, especially because of its UV-reflective nature. Responses in *Arabidopsis* have been studied after supplementing into soil and artificial media of liquid and solid nature at different concentrations. Majority of the works reported reduction in growth expressed as low percentage of seed germination and the reduction in biomass contributed by the decrease in number of leaves, rosette size and the length of primary and the lateral roots (Lee et al. 2010; Landa et al. 2012; Landa et al. 2015; Wang et al. 2016a, b; Nair and Chung 2017; Vankova et al. 2017; Yang et al. 2018). But these interferences are reported to be not due to their internalization, rather than the adsorption on to root surface. Anthocyanin induction and reduction in chlorophyll content were two visible changes induced, which are characterized as stress indicators (Wang et al. 2016a, b; Vankova et al. 2017). However, a lateral increase in the level of carotenoids was also noticed along with that (Wang et al. 2016a, b). Reduction in the rate of photosynthesis, transpiration and conductance of leaf stomata were few other notable physiological changes observed. Increase in the concentration of Zn was experienced in tissues, which in turn affected the nutrient homeostasis resulting in reduction of total concentrations of K, S and Cu (Nair & Chung, 2017). Hormonal changes including increase of cytokinin in roots, ABA in leaves and apex, reduction of Zeatin and IAA in apex, etc. have been induced by ZnONPs (Vankova et al. 2017). This hormonal imbalance was accompanied with the reduction of jasmonic acid and jasmonate isoleucine in apex. Gene expression changes observed were somewhat similar to the other types of nanoparticles discussed earlier. Genes related to stress response, especially oxidative stress response, signal transduction, hypoxia, detoxification, wound response and defence, metal ion transport and homeostasis were found expressed in excess (Landa et al. 2012; Landa et al. 2015; Wang et al. 2016a, b; Nair and Chung 2017). Reduction of the rate of photosynthesis observed in few studies were later proved as due to the downregulation of genes involved in chlorophyll synthesis, photosystem I and electron transport (Landa et al. 2012; Wang et al. 2016a, b). Growth inhibition observed especially at the apices was also effected by the downregulation of the genes functioning in microtubule synthesis and protein translation in addition to the above-mentioned ones (Landa et al. 2012). ZnONPs also increased the frequency of homologous recombination and induced epigenetic changes which was tested and confirmed using transgenic *Arabidopsis* (Yang et al. 2018). Since entry of ZnONPs into the plant cells is prevented, it cannot be considered that the particles can induce the wide variety of responses we have seen (Nath et al. 2018). Based on the results of the studies conducted along with Zn<sup>+</sup> ions, it can be concluded that the responses are of the Zn<sup>+</sup> ions liberated into the growth media from the particles (Yang et al. 2018). However, it has been shown that the adsorption of the particles to root surface can induce signal transduction pathways which can trigger an array of changes in the plant (Landa et al. 2012 & Landa et al. 2015).

**Iron:** Iron oxide nanoparticles (FeONPs) are used in drug delivery, magnetic resonance imaging, groundwater treatments, photocatalytic reactions, environmental remediation, etc. Interaction with *Arabidopsis* demonstrated inhibitory effects at high concentrations expressing morphologically as reduction in growth rate (Marusenko et al. 2013; Bombin et al. 2015). As observed in the case of some other nanoparticles, lower concentrations produced promotive effects (Kim et al. 2014). Native particles were not observed to be transported into the plant, but their charged counterparts were found distributed in roots and different parts of the shoot including stem, leaves, flowers and seeds (Bombin et al. 2015). Reduction in chlorophyll (Marusenko et al. 2013), pollen viability, pollen tube length and number of seeds (Bombin et al. 2015) was also observed. Growth promotion at lower concentrations is observed to be happening in different ways.  $H_2O_2$  formed on FeONP exposure induces loosening of cell wall, resulting in reduction in cell wall thickening, reorientation of microfibrils and increased incidence of endocytosis (Kim et al. 2014). Cell wall loosening has also increased leaf surface area (Kim et al. 2015). Increase in the activity of the plasma membrane  $H^+$  ATPase activity due to the reduction of apoplastic pH increased stomatal opening facilitating increased intake of  $CO_2$  without encountering excess water loss (Kim et al. 2015). Notwithstanding that *A. thaliana* offers the possibility of an in-depth investigation, especially for understanding the interactions at genetic level, none of the studies have attempted that.

**Carbon:** Carbon nanomaterials are available in different forms as fullerenes, nano-onions, nano-cones, nano-horns, carbon dots, carbon nanotubes, nano-beads, nano-fibres, nano-diamonds, and graphene. They are different in structure, size and shape and hence find applications in diverse fields like electronics, optics, nanomedicine, biosensors, renewable energy production, environmental remediation and as carriers for delivering molecules, metals, atoms, etc. Their responses in *Arabidopsis* have not been investigated in detail so far. Those that were studied in the plant are of carbon nanotubes (CNTs) examined at the cellular level. Single-walled carbon nanotubes (SWCNTs) were observed to be entering protoplasts and mesophyll cells (Shen et al. 2010; Yuan et al. 2012). They induce ROS, chromatin condensation and DNA breakage and result in cell death. ROS evolution confirmed by staining and expression analyses of the concerned genes is assumed to be inducing apoptosis (Shen et al. 2010). Multiwalled nanotubes (MWCNTs) also have somewhat similar effects, denoted by the reduction in cell viability, chlorophyll content, etc. (Lin et al. 2009). Both SWCNTs and MWCNTs form aggregates in media and inside the cells. Smaller aggregates of MWCNTs were found more inhibitory in effect than the larger ones (Lin et al. 2009).

**Cerium:** Cerium dioxide nanoparticles ( $CeO_2$ NPs) are used as a polishing material, additive in glass and ceramic, fuel cell material, in agricultural products and automotive industry. Their interaction with *Arabidopsis* has been investigated to some extent.  $CeO_2$ NPs at high concentrations inhibit growth, observed as redox ion in biomass of shoot and root (Yang et al. 2017). However, concentrations below 500 mg/L showed increase in biomass (Tumburu et al. 2017; Wu et al. 2018). Particles were found distributed in leaves and even inside chloroplast (Wu et al. 2017; Yang et al. 2017; Wu et al. 2018). Negatively charged particles enter

easily through their interaction with the positively charged plasma membrane (Wu et al. 2017 & Wu et al. 2018). Increase in  $H_2O_2$ , MDA and the associated reduction in chlorophyll with respect to  $CeO_2$ NPs treatment designates toxicity development in the plant (Yang et al. 2017). On the other hand, negatively charged  $CeO_2$ NPs have opposite effects. They are capable of scavenging free radicals and thereby support to survive under stress (Wu et al. 2017 & Wu et al. 2018). These particles induce upregulation of the genes involved in transcription, ageing,  $H_2O_2$  regulation, cell cycle, stress responsive genes in shoot which is accompanied by downregulation of auxin stimulus and cell wall modification (Tumburu et al. 2017). In roots, genes functioning in transcription, phenyl propanoid metabolism, seed maturation, and response to GA were upregulated and those of cell wall formation, syncytium formation, cell signalling, cell cycle and polysaccharide catabolism were downregulated. In essence,  $CeO_2$ NPs are identified as toxic. Experiments conducted with bulk  $CeO_2$  along with  $CeO_2$ NPs proved the ineffectiveness of  $CeO_2$  ions in inducing toxic changes equivalent to the  $CeO_2$ NPs (Yang et al. 2017). Hence it cannot be assumed that the Ce ions released from disintegrating nanoparticles in suspension are responsible for the changes.

**Titanium:** Titanium dioxide is the ingredient imparting white tint to almost all products used in industry, medicine, cosmetics, etc. Their range of applications widened as the size of the particles narrowed down. As a result, titanium dioxide nanoparticles ( $TiO_2$ NPs) emerged as the one produced in the largest quantity worldwide and has resulted in its release in large amount into the environment. *Arabidopsis* has been used to characterize the effects in plants brought about by the particles. Almost all studies done up to date are carried out either in solid or liquid growth media. Conjugation with the dye alizarin deduced their entry into almost all parts of the plant body (Kurepa et al. 2010). Lower concentrations promoted growth evidenced as increase in germination rate and biomass (García-Sánchez et al. 2015; Liu et al. 2017). Whereas the effect was opposite in higher concentrations bringing about reduction in biomass and yield (Liu et al. 2017). Prominent physiological impacts noticed were increase in chlorophyll and total protein at lower concentrations and their reversal upon treatment with higher concentrations. Higher concentrations elevated the activity of antioxidant enzymes SOD, CAT, POD and APX depicting oxidative stress (Liu et al. 2017). Reduction in chlorophyll, increase in oxygen and vitamin E production were also observed in case of higher concentrations. Chlorophyll breakdown results in phytol formation, which is being utilized for vitamin E production by *Vte5* gene product (Szymańska et al. 2016). Experiments with Alizarin conjugated particles identified that the particles disrupt microtubule network of the cell, which was characterized by the reduction in alpha tubulin, beta tubulin and ubiquitin with a corresponding increase in ubiquitinated proteins (Wang et al. 2011). Increase in light absorption, fluorescence quantum yield of chloroplast, electron transfer, photolysis, oxygen evolution and hill reaction on exposure to low concentrations enhance the efficiency of photosynthesis and hence can be suggested for enhancement of the process of carbohydrate fixation (Ze et al. 2011). Expression profiling techniques have identified the genes functioning in light absorption, photosynthesis (Ze et al. 2011; Tumburu et al. 2015, 2017), nutrient

transport (García-Sánchez et al. 2015; Tumburu et al. 2017), root development and growth (García-Sánchez et al. 2015; Tumburu et al. 2015, 2017), stress response and hormone response (Tumburu et al. 2015, 2017) gets differentially regulated during exposure to TiO<sub>2</sub>NPs. Gene expression changes accompanied with morphological and physiological changes were upregulation of tocochromanol biosynthesis gene which utilizes phytol formed during the degradation of chlorophyll (Szymańska et al. 2016), downregulation of GST and GR with a corresponding increase in the activities of SOD, CAT, POD and Ascorbate Peroxidase (APX) in roots (Liu et al. 2017). Overall, TiO<sub>2</sub>NPs enhance the light capturing capability and increase the efficiency of photosynthesis and hence beneficial to plants. However, we cannot conclude as beneficial in all respects because of the demonstration of breakdown of chlorophyll and the rupture of microtubule network associated.

**Quantum Dots:** These particles are mostly employed in electronic industry because of their electrical and luminescence properties. Some of them are also used for labelling or staining applications in biology. They are prepared as binary compounds such as lead sulphide, lead selenide, cadmium selenide, cadmium sulphide, cadmium telluride, iridium arsenide and iridium phosphite. Even though they are identified as toxic to animal system because of their small size, photolytic activity, high surface reactivity and mechanical stability, very few of them have been investigated for phytotoxicity, especially in Arabidopsis. Exposure of the plant to cadmium quantum dots formed in combination with selenium, sulphur and zinc produced differential responses according to the concentration, type of treatment and its duration. Cadmium sulphide quantum dots (CdS QDs) in high concentration inhibited seed germination and root growth (Marmioli et al. 2014; 2020). Inhibition of the root growth was preceded by swelling and bursting which is assumed to be due to the clogging of the vasculature due to aggregation of the particles. Appearance of chlorosis, necrotic regions, increased wax deposition and reduction in stomatal and trichome density on leaves clearly indicated toxicity. Overall reduction in respiration was noticed. Increase in the biosynthesis of phenolic compounds, glutathione, antioxidant activity and lipid peroxidation indicated evolution of ROS. Predominant increase in the expression of genes associated with heat shock, temperature stress regulation, ROS metabolism and ion transport reinforced the assumption. Comparison of the effects with that of CdSO<sub>4</sub> helped to differentiate the specific effects of CdS QDs (Marmioli et al. 2014, 2020). For understanding the role of surface charge in uptake and transport, cadmium selenide QDs (CdSe QDs) were coated with charged molecules and supplied to plant through liquid growth medium (Koo et al. 2015a, b). Charged particles observed to be moving fast through the vascular tissue and getting distributed into almost all parts of the plant. On the other hand, much of the neutral QDs formed aggregates and found lodged at short distances. Finally, anionic particles were localized in the apoplast and the cationic ones intracellular. Anionic particles induced chlorosis and their cationic counterparts produced browning of the leaves. Hence experimental evidences indicate that surface properties have determinant role in the uptake and transport of QDs. Experiments with CdSe QDs coupled with salicylic acid binding moiety were found successful in internalization and proved the utility of modifying surface properties for intracellular targeting (Liu et al. 2015).

Zinc selenide QDs (ZnSe QDs) when used at two different concentrations (100 & 250  $\mu\text{M}$ ) exhibited different responses (Kolackova et al. 2019). Increase in gallic acid, phytochelatin, phenols,  $\text{H}_2\text{O}_2$  scavenging and antioxidant activity were induced by the lower dose, and the higher one was characterized by a reduction in membrane lipidemic compounds,  $\text{H}_2\text{O}_2$  scavenging, and antioxidant activity. Expression of genes in phytochelatin biosynthesis found upregulated in case of 100  $\mu\text{M}$  and the genes in glutathione synthesis and ROS scavenging in 250  $\mu\text{M}$  group. Overall, quantum dots at higher concentrations are inhibitory to plant growth and development.

## 2 Conclusion and the Future Perspectives

Seed germination and the changes in growth pattern of root and shoot are considered for the assessment of the effects due to exposure to nanomaterials. The parameters used are not reliable because of the lack of phenotypes for most of the changes occurring at molecular level. Results vary depending upon concentration, size, shape, surface charge and the chemical composition of the nanomaterials tested. Hence, it is important to carry out nanoparticle testing with the parameters for assessing changes in physiology at molecular level. Nanomaterials at low concentrations show growth promotion. In case of FeONPs, the changes associated with growth enhancement were persuaded by the  $\text{H}_2\text{O}_2$  generated inside.  $\text{H}_2\text{O}_2$  induces loosening of cell wall and reduction of cell wall thickening, reorientation of microfibrils and increased incidence of endocytosis (Kim et al. 2014). Cell wall loosening also results in an increased leaf surface area (Kim et al. 2015). Reduction in apoplastic pH increases the activity of the plasma membrane  $\text{H}^+$  ATPase activity which, in turn, augment stomatal opening facilitating increased intake of  $\text{CO}_2$  without encountering excess water loss (Kim et al. 2015). Growth enhancement can also happen through the improvement of the efficiency of carbohydrate fixation as observed in case of  $\text{TiO}_2$ NPs (Ze et al. 2011). Thus, the mode of operation of growth enhancement is different in case of different nanomaterials and demands more extensive studies needed to figure out the details. On the contrary, exposure to high concentration of nanoparticles induces stress characterized by the production of anthocyanin and inhibition of chlorophyll biosynthesis (Nair and Chung 2014b; Wang et al. 2016a, b; Vankova et al. 2017). Stress signalling is also mediated through ROS (Baghkheirati and Lee 2015; Wang et al. 2016a, b; Landa et al. 2017; Liu et al. 2017). Chlorophyll degradation is activated by the ROS developed as byproducts inside the chloroplast (Rogers and Munné-Bosch 2016). ROS can also damage a large variety of cellular biomolecules, including carbohydrates, nucleic acids, lipids, proteins, etc., and alter their functions. Antioxidant enzymes at this point prevent the burst in ROS level and prevent sudden cell death. This is accompanied with the changes in the GSSG/GSH and MDA pool (Hasanuzzaman et al. 2019). Anthocyanin having antioxidant activity is also produced in excess to act against ROS (Zhang et al. 2012). Changes in gene expressions are known in case of some nanomaterials which facilitate to monitor the effects at molecular level and to establish the link between the pathways of signal perception and response.

Nanomaterials can enter the plant body through different routes because of their presence in air, water and soil. In majority of the studies, the nanomaterials tested have been applied into the growth medium owing to their absorption through the root system. Uptake through aerial parts, mainly through openings like stomata need to be considered and application methods including foliar spray should be examined (Kolackova et al. 2019). As the mode of translocation will be different in this case, its impact with respect to the root to shoot translocation should be compared. Arabidopsis being a plant with small, slender vasculature, the possibilities for in vivo localization or accumulation remain unexplored for majority of nanomaterials. Another important limitation of the studies conducted so far is the short period of exposure, mostly done for 2–3 weeks. That kind of an experimental set-up cannot mimic the actual conditions prevalent in any ecosystems. The short life cycle of Arabidopsis is ideal for characterizing the responses at different stages of growth and few studies have explored these possibilities (Ke et al. 2018). The type of nanomaterials and their volume released into the environment is different according to their cost of production and usage. For instance, the production of TiO<sub>2</sub>NPs is higher with respect to AuNPs. Accordingly, we can expect a higher percentage of TiO<sub>2</sub>NPs in the environment than the latter. Stability of nanomaterials in the environment should also be verified. They can interact with other chemical moieties in air, water and soil and get transformed to higher reactive or toxic forms. This can also happen once they are inside the plant. Many nanomaterials form aggregates in plants and it will be interesting to know whether the conditions for aggregate formation are induced by plant molecules to prevent their movement and reactivity. The important advantages of Arabidopsis as an experimental plant are the availability of its sequenced genome and of gene overexpression and mutant lines. Nevertheless, gene expression studies at the transcriptomic level have been conducted in response to nanomaterial exposure; few attempts have been made to testify the effects using gene overexpression and mutant lines (Yang et al. 2018). Hence, we can expect more realistic studies in Arabidopsis to characterize the effect of nanomaterials in future.

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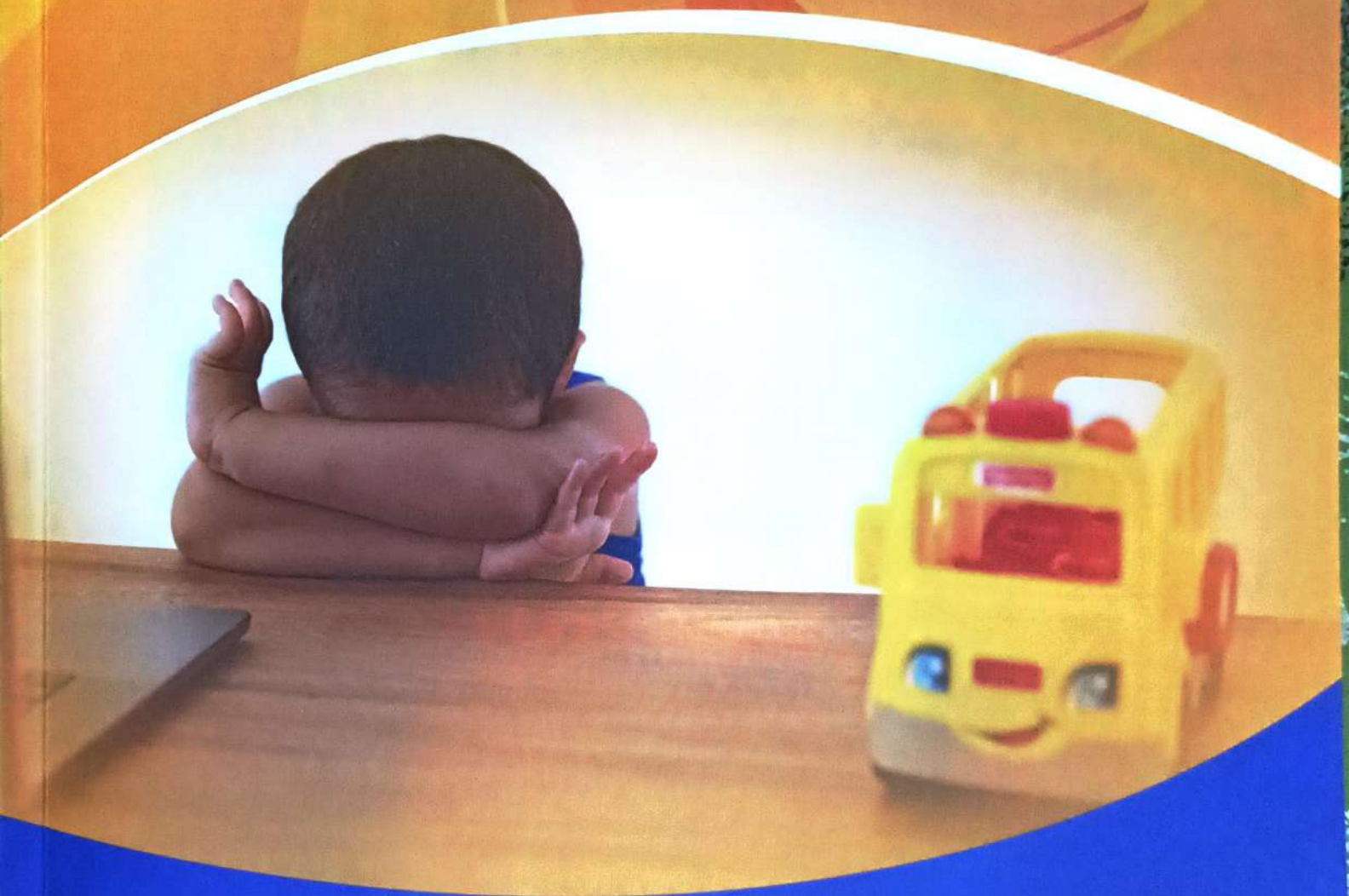
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